UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
F. EDWARD HÉBERT SCHOOL OF MEDICINE
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4799

March 7, 2007

BIOMEDICAL GRADUATE PROGRAMS

Ph.D. Degrees

Interdisciplinary
-Emerging Infectious Diseases
-Molecular & Cell Biology
-Neuroscience

Departmental
-Clinical Psychology
-Environmental Health Sciences
-Medical Psychology
-Medical Zoology
-Pathology

Doctor of Public Health (Dr.P.H.)

Physician Scientist (MD/Ph.D.)

Master of Science Degrees

-Molecular & Cell Biology
-Public Health

Masters Degrees

-Comparative Medicine
-Military Medical History
-Public Health
-Tropical Medicine & Hygiene

Graduate Education Office
Dr. Eleanor S. Metcalf, Associate Dean
Janet Anastasi, Program Coordinator
Tanice Acevedo, Education Technician

Web Site
www.usuhs.mil/geo/gradpgm_index.html

E-mail Address
graduateprogram@usuhs.mil

Phone Numbers
Commercial: 301-295-9474
Toll Free: 800-772-1747
DSN: 295-9474
FAX: 301-295-6772

APPROVAL SHEET

Title of Dissertation: "Biobehavioral Correlates of Depression in Reaction to Mental and Physical Challenge"

Name of Candidate: Ali Weinstein
Doctor of Philosophy Degree
29 March 2007

Dissertation and Abstract Approved:

David Krantz, Ph.D. — 3-29-07 Date
Department of Medical & Clinical Psychology
Committee Chairperson

Willem Kop, Ph.D. — 4/12/07 Date
Department of Medical & Clinical Psychology
Committee Member

Tracy Sbrocco, Ph.D. — 4/12/07 Date
Department of Medical & Clinical Psychology
Committee Member

Patricia Deuster, Ph.D. — 4/12/07 Date
Department of Military & Emergency Medicine
Committee Member

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
MAR 2007		**00-00-2007 to 00-00-2007**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Biobehavioral Correlates of Depression in Reaction to Mental and Physical Challenge	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	8. PERFORMING ORGANIZATION REPORT NUMBER
Uniformed Services University of the Health Sciences,F. Edward Hebert School of Medicine,4301 Jones Bridge Road,Bethesda,MD,20814-4799	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Depression is the most common mental disorder in the United States. Individuals with depression are at an increased risk of cardiovascular morbidity and mortality. However, little is known regarding the possible mechanisms to explain this relationship. One of the possible pathways includes exaggerated responsiveness to challenge in depressed individuals. The overarching hypothesis of this investigation is that depressed individuals display higher reactivity to mental and physical challenge than non-depressed controls. Specifically, it was examined whether elevated neurohormonal and negative mood responses to challenge tasks would result in elevated cardiovascular and inflammatory responsiveness. Reactivity to mental (mental arithmetic and anger recall) and physical challenge (exercise bout on treadmill) was assessed in 14 depressed and 16 non-depressed control participants. Neurohormonal (adrenocorticotropic hormone, cortisol, norepinephrine, and epinephrine), negative mood, cardiovascular (systolic blood pressure, diastolic blood pressure, and heart rate), and inflammatory (IL-6, TNF-a, and CRP) responses to mental challenge (anger recall and mental arithmetic) and physical challenge (treadmill exercise) tasks were assessed. Results indicated that depressed participants: 1) displayed higher reactivity of neurohormonal and negative mood measures, as well as increased cardiovascular and inflammatory responses during the challenge tasks; 2) neurohormonal and negative mood responsiveness were associated with cardiovascular and inflammatory reactivity; and 3) these relationships displayed variability across measures and challenge tasks. This study demonstrates that hyper-reactivity to challenge tasks can be documented among depressed individuals. Future research is needed to determine the consequences of hyperreactivity to the development of adverse cardiovascular health outcomes. These findings may also lead to novel interventions aimed at reducing hyper-reactivity to challenge with potential positive effects on quality of life for individuals with depression.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**110**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

COPYRIGHT STATEMENT

The author hereby certifies that the use of any copyrighted material in the dissertation manuscript entitled:

"Biobehavioral Correlates of Depression in Reaction to Mental and Physical Challenge"

Is appropriately acknowledged and, beyond brief excerpts, is with the permission of the copyright owner.

Ali A. Weinstein
Department of Medical and Clinical Psychology
Uniformed Service University of the Health Sciences

ABSTRACT

Title of Thesis: Biobehavioral Correlates of Depression in Reaction to Mental and Physical Challenge

Author: Ali A. Weinstein, Doctor of Philosophy, 2007

Thesis directed by: Willem J. Kop, Ph.D.
Associate Professor
Department of Medical and Clinical Psychology

Depression is the most common mental disorder in the United States. Individuals with depression are at an increased risk of cardiovascular morbidity and mortality. However, little is known regarding the possible mechanisms to explain this relationship. One of the possible pathways includes exaggerated responsiveness to challenge in depressed individuals. The overarching hypothesis of this investigation is that depressed individuals display higher reactivity to mental and physical challenge than non-depressed controls. Specifically, it was examined whether elevated neurohormonal and negative mood responses to challenge tasks would result in elevated cardiovascular and inflammatory responsiveness.

Reactivity to mental (mental arithmetic and anger recall) and physical challenge (exercise bout on treadmill) was assessed in 14 depressed and 16 non-depressed control participants. Neurohormonal (adrenocorticotropic hormone, cortisol, norepinephrine, and epinephrine), negative mood, cardiovascular (systolic blood pressure, diastolic blood pressure, and heart rate), and inflammatory (IL-6, TNF-α, and CRP) responses to mental challenge (anger

recall and mental arithmetic) and physical challenge (treadmill exercise) tasks were assessed.

Results indicated that depressed participants: 1) displayed higher reactivity of neurohormonal and negative mood measures, as well as increased cardiovascular and inflammatory responses during the challenge tasks; 2) neurohormonal and negative mood responsiveness were associated with cardiovascular and inflammatory reactivity; and 3) these relationships displayed variability across measures and challenge tasks. This study demonstrates that hyper-reactivity to challenge tasks can be documented among depressed individuals. Future research is needed to determine the consequences of hyper-reactivity to the development of adverse cardiovascular health outcomes. These findings may also lead to novel interventions aimed at reducing hyper-reactivity to challenge with potential positive effects on quality of life for individuals with depression.

Biobehavioral Correlates of Depression

in Reaction to Mental and Physical Challenge

by

Ali A. Weinstein

Dissertation submitted to the faculty of the

Department of Medical and Clinical Psychology

Graduate Program of the Uniformed Services University

of the Health Sciences in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy,

2007

Dedication

This work is dedicated to the memory of my grandmother, Helen Sarah Fox. She approached life with a perseverance and resiliency that I always respected and tried to emulate. The completion of this dissertation is an accomplishment made possible by the unconditional love and support I received from her.

Thank you Grandma – I love you!

Acknowledgements

I would like to express my sincere appreciation to a number of individuals who helped me through this process. First, I would like to thank my advisor, Dr. Willem Kop, for his continuous support and guidance. His dedication to the advancement of my education has been unyielding and compassionate. I will always be proud to be his first student. Dr. David Krantz provided critical and thoughtful evaluations of this project as the committee chairperson. Dr. Sbrooco offered critical guidance on the clinical aspects of this project, which should never be forgotten. Also, she was crucial in my job search process by providing advice and counsel. In addition, Dr. Patricia Deuster was extremely helpful in the planning and implementation of the exercise portion of this project. Dr. Deuster never refused a single request and was always available to discuss the project with me. In addition, she allowed me the use of the Human Performance Laboratory to conduct the research project.

I would like to acknowledge Elaine Cornell and Professor Russell P. Tracy at the University of Vermont and Milburn Emory and Professor Robert Bonsall at Emory University for their guidance and tireless efforts on the blood assays.

Finally, I would like to thank all of the individuals who helped me run the participants through the protocol, as it was a team effort. Dr. Jennifer Francis, Dr. Charles Beadling, Nicole Fendrick, Stacey Zeno, Angie Demoncada, Jessica Eng, Emily Lichvar, Emily Gross, Stephanie Garey, and Shannon Branlund were all instrumental in the completion of this project and I thank them for all of their hard work.

Table of Contents

List of Tables

List of Figures

Introduction

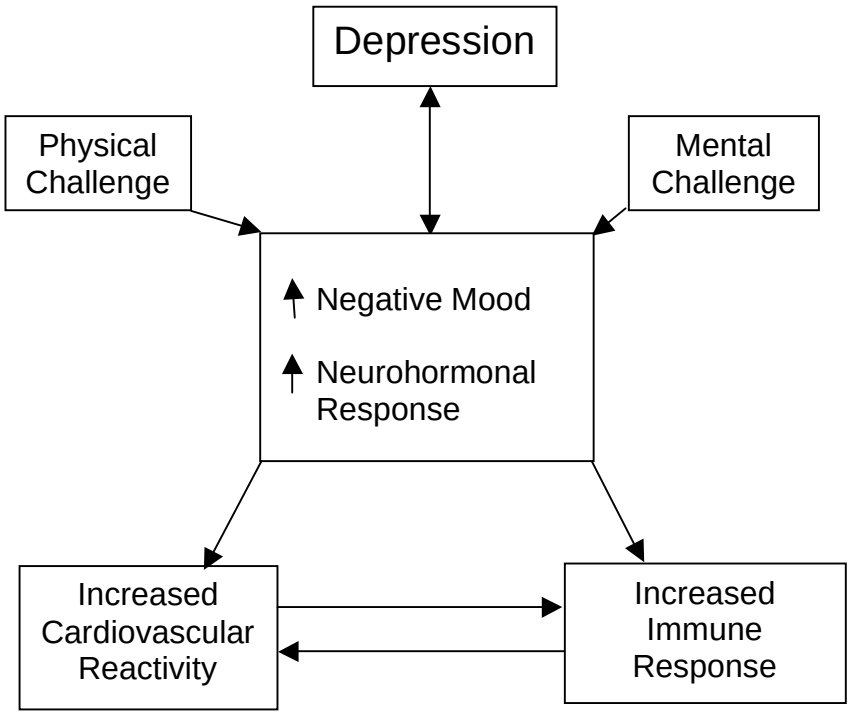
Depression is the most common mental disorder in the United States with prevalence rates ranging from 3.3% to 17.1% (Kessler et al., 1994). Biological and physiological correlates of depression include changes in neurohormonal parameters (e.g., increased hypothalamic-pituitary-adrenal axis activity), cardiovascular alterations (e.g., increased ambulatory blood pressure), and inflammatory markers (e.g., elevated pro-inflammatory cytokines). These biological and physiological correlates of depression are also known to increase in response to both mental and physical challenge tasks. Little is known, however, about these responses to challenge in individuals with depressive mood disorder. These responses to challenge may be important in explaining the increase in mortality and morbidity in individuals with depression.

The specific goal of the current investigation was to examine the acute effects of mental and physical challenges among depressed participants compared to non-depressed participants in terms of mood, cardiovascular, and neuroimmunological reactivity. The overarching hypothesis of this investigation was that the depressed individuals would display more negative mood and increased neurohormonal responses to physical exercise and mental challenge, resulting in elevated cardiovascular and inflammatory responsiveness. Figure 1 provides a model of the conceptual framework of this study.

The following sections provide an overview of (1) the definition and prevalence of depression; (2) neurohormonal and emotional concomitants of

depression; (3) cardiovascular characteristics of depression; (4) immune system parameters in depression; and (5) health behavior correlates of depression. These sections provide a selective review of the evidence supporting the underlying biopsychological theory of this dissertation (Figure 1).

Figure 1.



Background

I. Definition and Prevalence of Depression

a. Definition

The Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) is the most commonly used nomenclature for classifying psychiatric disorders in the United States (American Psychiatric Association, 2000). The DSM-IV criteria for Major Depressive Disorder (MDD) require the presence of at least five out of nine symptoms: depressed mood, loss of interest in normally pleasurable activities (anhedonia), changes in sleep (increase or decrease), changes in appetite or weight (increase or decrease), fatigue, change in psychomotor activity (increase or decrease), feelings of guilt or worthlessness, difficulty thinking or concentrating, or recurrent thoughts of death and/or suicidal plans or attempts. To qualify for DSM-IV MDD criteria, at least one of the first two symptoms must be present (depressed mood and/or anhedonia). Furthermore, these symptoms must have a duration of greater than 2 weeks and interfere substantially with patients' daily functioning (American Psychiatric Association, 2000).

b. Prevalence

Estimates of the lifetime prevalence of MDD in community-based adult samples vary considerably across studies, ranging from 3.3% (Lee et al., 1990) to 17.1% in the US (Kessler et al., 1994). These differences may be explained to a large extent by methodological aspects of the investigations, such as the specific diagnostic classification system used to define MDD, the diagnostic interview or questionnaire employed, and the age, gender, and sociodemographic composition of the study sample (Kessler et al., 1994).

Most depressive episodes last longer than the minimum diagnostic requirement of 2 weeks, with the average episode lasting 6 to 8 months (Lehmann, 1983; Mueller & Leon, 1996). Furthermore, MDD tends to be episodic; a majority of people who recover from a MDD episode will have a recurrence of the disorder (Lehmann, 1983).

The debilitating effects and substantial costs of MDD for individual patients as well as society have received much scientific and political attention. For example, in the Epidemiological Catchment Area (ECA) study, MDD was associated with increased use of general medical services, increased use of emergency departments for emotional problems, impaired physical and mental health, lost time at work, and increased rates of attempted suicide (Johnson et al., 1992). The Medical Outcomes Study (MOS) also found that individuals with either MDD or sub-clinical depressive symptoms reported decreased well-being and functioning when compared with those without chronic health problems, and

comparable or worse functioning than patients with major chronic medical disorders such as diabetes mellitus, hypertension, and arthritis (Wells et al., 1989). The economic burden of depression in the US (including direct treatment costs and indirect costs associated with depression in the workplace) has been estimated at $83.1 billion per year (Greenberg et al., 2003). Thus, MDD is a prevalent and recurrent disorder, associated with high rates of morbidity, poor quality of life, and significant economic costs.

Depression is more prevalent among patients with medical disorders compared to healthy individuals (Ormel et al., 1994), and adversely affects clinical outcomes of co-morbid medical conditions (Coulehan et al., 1997; Spitzer et al., 1994). Rowe and colleagues (1995) surveyed 1898 patients attending a variety of primary care clinics and reported that 21.7% of women and 12.7% of men met criteria for depression during the previous 30 days. Lifetime estimates ranged from 36.1% for women to 23.3% for men (Rowe et al., 1995). Other studies have estimated the prevalence of major depression in primary care outpatients in the range of 20% (Barrett et al., 1988; Schulberg & Burns, 1988) to 30% (Kamerow, 1988).

Numerous studies indicate that patients with depression and comorbid medical illness have greater impairment of daily functioning, poorer quality of life, and more disability and/or lost productivity because of illness than non-depressed patients with identical illnesses (Ormel et al., 1994; Spitzer et al., 1994). Depression is associated with an increased risk of total mortality (Wulsin et al., 1999), morbidity and mortality in patients with coronary artery disease (Kop

& Ader, 2001; Lesperance et al., 2002), heart failure (Ramasubbu & Patten, 2003), renal failure (Kimmel et al., 2000; Lopes et al., 2002), and cancer (Valente & Saunders, 1997). Depressed patients with diabetes mellitus have poorer glycemic control and elevated rates of diabetic complications compared to diabetic patients without depression (de Groot et al., 2001; Lustman et al., 2000). Thus, depression is not only more prevalent among patients with medical conditions, but also predicts adverse progression of these conditions. The present study investigated depression in otherwise healthy individuals in order to minimize the potential confounding effects of medical comorbidity on cardiovascular and neuoroimmunological measures.

II. Neurohormonal and Emotional Concomitants of Depression

Depression is associated with various biological (e.g., neurohormonal activity) and emotional response characteristics (Figure 1). These neurohormonal and emotional response characteristics (discussed in detail in subsequent sections) among depressed individuals may have important implications for cardiovascular and immune-system related responses to acute challenges such as mental arousal and physical exercise. In this dissertation, neurohormones are defined as hormones that affect the brain. It is a generic term used to encompass the hormonal variables of interest in the present work: ACTH, cortisol, norepinephrine, and epinephrine.

a. Neurohormonal Correlates of Depression

Hypothalamic-pituitary-adrenal axis. In depressed patients, disrupted hypothalamic-pituitary-adrenal (HPA) axis function involves increased central nervous system drive (increased cortisol secretion), impaired negative cortisol feedback, and hypertrophy of the adrenal gland. The principal neurohormonal characteristic in depressed patients involves increased cortisol secretion from the adrenal cortex. This change is present in approximately 50% of patients with MDD (Halbreich et al., 1985; Pfohl et al., 1985). Patients with atypical features of depression (approximately 15-40% of MDD patients) often present with a divergent neurohormonal profile (i.e., decreased cortisol secretion) (Gold et al., 2002). In addition to the core symptoms of typical MDD, atypical depression is defined by the ability to temporarily feel better in response to a positive life event, plus any two of the following criteria: hypersomnia (excessive sleep), hyperphagia (overeating), a feeling of heaviness in the limbs, and/or a sensitivity to rejection (American Psychiatric Association, 2000). This review will primarily address neurohormonal correlates of typical MDD, atypical depression was assessed as a potential effect-modifying variable.

Autopsy studies on depressed individuals who committed suicide have revealed a 4-fold increase in the number of cells expressing corticotrophin releasing hormone (CRH) in the paraventricular nucleus (PVN) of the hypothalamus, and a 3-fold increase in the co-expression of CRH and arginine vasopressin (AVP) in the PVN (Raadsheer et al., 1994). Other evidence for an

increased central drive include reports that the HPA axis overrides the effects of cortisol synthesis inhibition by metyrapone (Young et al., 1994). The increased central drive to the HPA axis in depression could be mediated by either CRH or by AVP or by both (Checkley, 1996).

Impaired negative feedback in control of the HPA axis by corticosteroids has also been observed in depressed patients. The dexamethasone suppression test has been designed to assess delayed negative feedback at glucocorticoid receptors (GRs). The dexamethsaone suppression test is abnormal in many patients with MDD, showing impaired suppression of adrenocorticotropic hormone ACTH (Carroll et al., 1981). The inhibition of ACTH in response to an infusion of hydrocortisone is a test of fast-feedback inhibition at the hippocampus level, and ACTH suppression is impaired in depression (Carroll et al., 1981).

Another change in the HPA axis is hypertrophy of the adrenal gland. In depressed patients, hypertrophy of the adrenal gland has been demonstrated by CAT scan studies (Nemeroff et al., 1992), by MRI (Rubin et al., 1995), and at post mortem examination (Dorovini-Zis & Zis, 1987). An enlargement of pituitary gland volume in depression has also been demonstrated (Krishnan et al., 1991). Thus, most evidence indicates increased central drive of the HPA axis, an impaired negative feedback system of the HPA axis, and hypertrophy of the adrenal gland among individuals with MDD. As a result, depressed patients sustain an increased secretion of corticosteroid hormones and are able to display an additional corticosterone response to superimposed stress (Checkley, 1996).

Several studies have shown that depressed patients release significantly more cortisol after CRH stimulation in comparison with age-matched control participants (Gold et al., 1987). Depressive disorders are associated with sustained HPA axis activation resulting in higher than normal levels of plasma cortisol under basal conditions (Maes et al., 1991). This hypercortisolistic state has been suggested to cause hippocampal defects, which may impair the ability to terminate increased glucocorticoid production/levels, which in turn, may give rise to higher than normal cortisol release to a challenge test (van Cauter et al., 1996).

Sympathoadrenal system. In addition to hyperactivity of the HPA axis, depressed individuals also have an overactive sympathoadrenal system (SAM) (Leonard, 2001). The adrenal medulla and sympathetic nervous system (SNS) together constitute the SAM. Epinephrine in plasma is derived from the adrenal medulla, whereas plasma norepinephrine (NE) concentrations reflect secretion that originates largely from sympathetic nerve terminals, with the remaining NE provided by the adrenal medulla. Peripheral plasma NE concentrations are determined not only by the rate of release from sympathetic system nerve terminals, but also by reuptake into presynaptic terminals, local metabolic degradation, and redistribution into multiple physiologic compartments (Ressler & Nemeroff, 1999). Hypersecretion of NE in unipolar depression has been documented by elevated plasma NE and NE metabolite concentrations, as well as elevated urinary concentrations of NE and its metabolites. After treatment

with tricyclic antidepressants (TCAs), urinary excretion of NE and its metabolites diminishes together with plasma NE concentrations (Ressler & Nemeroff, 1999). Thus, SAM hyperactivity seems to represents a state rather than a trait marker of depression, possibly reflecting increased CRF release within the CNS (Leonard, 2001).

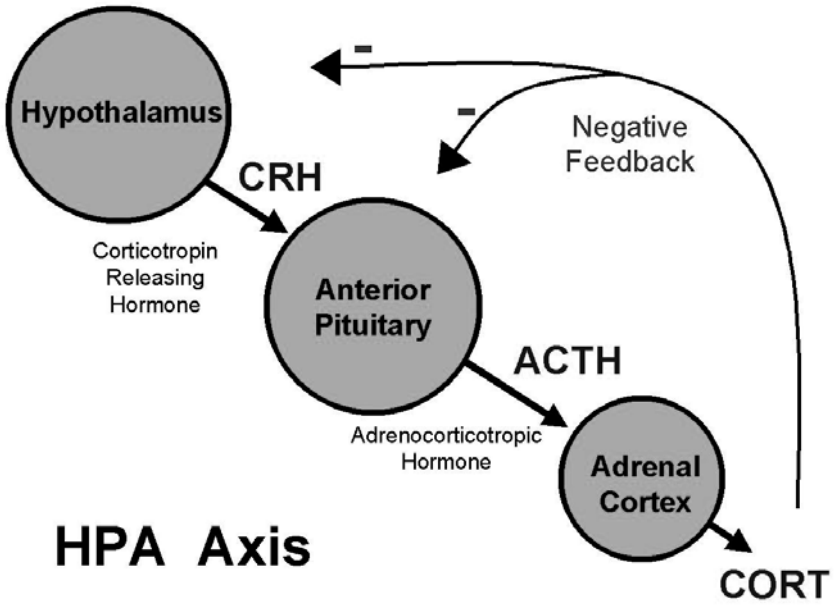
An important neurotransmitter involved in depression is serotonin (5-HT). A deficiency in brain serotonergic activity increases vulnerability to MDD (Maes & Meltzer, 1995). Disorders in serotonergic activity contribute to many of the symptoms of major depression, for example, mood, appetite, sleep, activity, suicide, sexual, and cognitive dysfunction. There is also evidence that interference with 5-HT synthesis or storage may induce depression in some vulnerable individuals (Maes & Meltzer, 1995). Finally, the commonly used anti-depressant medications act, at least in part, by enhancing central serotonergic activity (e.g., fluoxetine).

In contrast to NE, 5-HT is more commonly measured in cerebrospinal fluid than in plasma or urine because central serotonergic activity is specifically important to MDD (Leonard, 2000). Therefore, serotonergic activity will not be assessed in this investigation because of the invasive nature of evaluating cerebrospinal fluid and the lack of empirical support for the usefulness of measuring plasma 5-HT as related to mental stress and exercise responses. The present study assessed cortisol and ACTH as HPA axis indices and NE and epinephrine as markers of SAM and SNS activation.

b. Neurohormonal Responsiveness to Challenge

Several similarities have been documented between the acute stress response and major depression: both can be characterized by increased levels of neurohormones, blood pressure, and heart rate, as well as increased arousal and mobilization of energy stores. The stress response and depression involve critical brain structures including the locus coeruleus and the central nucleus of the amygdala, which are both innervated by corticotropin releasing factor (CRF)-containing nerve terminals. In depression, some aspects of the normal stress response seem to escalate to a pathologic state in which there is a failure to respond appropriately to usual counterregulatory feedback mechanisms (Figure 2), resulting in a sustained version of a usually transient phenomenon, i.e., hyperactivity of the HPA or SAM axis (Strohle & Holsboer, 2003).

Figure 2.



Neurohormonal response to mental challenge. Acute psychological stressors can activate the autonomic nervous system and promote the release of pituitary and adrenal hormones. A variety of experimental protocols for the induction of psychological stress in a laboratory setting have been evaluated for their physiological effects, including cardiovascular and sympathetic nervous system responses, HPA axis activation and changes in gonadal and neurohormones (growth hormones and prolactin) (Gerra et al., 1998; Mutti et al., 1989; Oltras et al., 1987; Krantz & Manuck, 1984).

Autonomic and neurohormonal activation in response to stressors serve to mobilize metabolic resources to support the requirements of "fight or flight." The stressors of contemporary society (e.g., traffic), however, often do not require or even allow behavioral fight or flight responses, and the autonomic and neurohormonal reactions to acute psychological stressors substantially exceed metabolic requirements.

Mental stress induced in the laboratory can produce a significant SNS response that differs in certain respects from the sympathetic response to physical stress. In this dissertation, stress is defined as "a state of physical or psychological strain which imposes demands for adjustment upon the individual" (Corsini, 2002). Mental stress induces larger increases in plasma epinephrine than NE whereas exercise induces larger increases in plasma NE than epinephrine. Furthermore, the rise in blood pressure in response to mental stress is more rapid than when compared with physical exercise. The mental

stress-induced increase in heart rate is smaller compared with physical exercise (Sims & Carroll, 1990).

Considerable evidence has now accumulated suggesting that sympathetic cardiac reactivity marks HPA activation to brief psychological stressors (Cacioppo et al., 1995; Kiecolt-Glaser et al., 1996; Lovallo et al., 1990; Uchino et al., 1995). These data, therefore, indicate that brief psychological stressors have an impact on the HPA axis especially when sympathetic cardiac reactivity is also high, possibly resulting from the common effects of the CRH system (Cacioppo et al., 1995).

Neurohormonal response to mental challenge in depression. In a recent study, participants with depressive symptoms demonstrated greater percent increases in plasma NE levels than participants with few or no depressive symptoms in response to the stress of a simulated speech on a recent anger arousing experience (Light et al., 1998). An ambulatory study found that an increase in depressive mood symptoms (caused by everyday stressors) was associated with increased 24-hour urinary norepinephrine (Grewen et al., 2004). The present study examined ACTH, cortisol, epinephrine, and NE responses to acute mental challenge (see Hypothesis 1.A).

Neurohormonal response to physical challenge. Muscular activity requires coordinated integration of many physiological and biochemical systems in order to maintain homeostasis. Neurohormones, as controlled by the endocrine

system, are one of the mechanisms used to regulate homeostasis. The endocrine response to an acute bout of exercise is characterized by a substantial increase in circulating catecholamines. The increase in norepinephrine is larger than the increase in epinephrine. Circulating catecholamine release is positively correlated with the intensity of exercise performed. As a person becomes more fit, the increase in catecholamines in response to intense exercise will diminish (Wilmore & Costill, 2001).

Acute exercise also increases ACTH and subsequently cortisol (Deuster et al., 2000). A positive relationship between intensity of exercise and increases in both ACTH and cortisol exists. The increase in ACTH and cortisol attenuates after an individual becomes more fit (Wilmore & Costill, 2001).

Neurohormonal response to physical challenge in depression. Depression is associated with increased plasma cortisol after completion of an acute bout of exercise (van der Pompe et al., 1999). Negative mood increases are positively correlated with cortisol release in MDD patients in response to an acute bout of exercise. It was hypothesized in the present study that the depressed participants would have an increased neurohormonal response to physical challenge than compared to participants without depression (see Hypothesis 1.A).

c. *Emotional Responsiveness to Challenge*

Mood response to mental challenge. Negative mood increases in response to mental challenge tasks and this response is associated with an increase in subjective feelings of stress (Puttonen et al., 2003). In a recent meta-analysis of acute mental stress tasks, it was found that participants responded to all of the tasks (mental arithmetic, star-tracing, and a speech task) with increases in negative emotion (Feldman et al., 1999). Meta-analytic statistical techniques were also used to assess the mean effect sizes (ES) for stressor-elicited changes in negative emotion on each task. The mean ESs were 0.74 on the speech task, 0.75 on the mental arithmetic, and 0.62 on the handgrip task. These are large and consistent increases in negative emotion across challenge tasks. Therefore, mental challenge tasks induce a stress response that is associated with an increase in negative mood.

Mood response to mental challenge in depression. The increase in negative mood to mental challenge tasks may be more exaggerated in depressed individuals. Diathesis-stress models of the development of depression propose that symptoms emerge whenever cumulative stressors exceed the individual's vulnerability threshold (Kovacs & Beck, 1978). Major life events and high expressed emotion have been found to precede the onset and recurrence of depression (Hooley et al., 1986; Kessler, 1997). Rather than reactions to the extreme exposures that life events often represent, sensitivity to

minor life events or daily hassles has been postulated to more closely resemble the underlying vulnerability for depression (Malla et al., 1990). A recent study showed that increased emotional sensitivity to small disturbances in daily life was present in patients with depression, indicating that altered stress-sensitivity may be a marker for depression (Myin-Germeys et al., 2001). Depressed patients also display larger increases in negative mood in response to daily stressors compared with control participants (2.5 ± 1.2 vs. 1.1 ± 0.3; p=0.0001) (Myin-Germeys et al., 2003). These increases in negative mood secondary to stressors may be partially explained by differing attributional styles used by the depressed individuals (depression is associated with a negative attributional style (Kwon & Laurenceau, 2002)).

Along with attributional style, appraisal of how stressful an event is can influence mood reactions to stressors (Folkman & Lazarus, 1988). Primary appraisals of stressors such as unpleasantness and stressfulness are associated with larger changes in mood (Marco et al., 1999; van Eck et al., 1998). Depressed individuals appraise events as more unpleasant and stressful compared to control participants (Beck et al., 1979). In a daily diary study, initial depression level was associated with more negative appraisals of events and less active coping (Gunthert et al., 2005). In addition to a more negative appraisal, depressed patients are more likely to blame themselves for their problems as compared to non-depressed controls (Walls & Hayes, 2000). Therefore, depressed patients may react with more negative mood because of

increased emotional sensitivity, negative appraisals, less active coping, or feelings of personal responsibility for the stressor itself.

It was hypothesized in this study that the depressed individuals would react to the mental challenge tasks with more negative mood compared to the control participants (see Hypothesis 1.B). The increase in negative mood may be caused by the differences in emotional sensitivity, attributional style, coping style, and/or appraisal of the challenge task. All of these factors have been shown to differentiate depressed individuals from their non-depressed counterparts (as discussed above). The current project focused on the negative mood changes to mental challenge and the subsequent effect on cardiovascular and inflammatory reactions.

Mood response to physical challenge. The research literature generally supports acute effects of exercise on mood, and over 85% of studies reported some degree of improved mood to an acute bout of exercise (Yeung, 1996). Exercise may therefore be a useful short-term strategy for alleviating psychological distress. In general, the mood effects of aerobic exercise occur irrespective of gender or age (Yeung, 1996).

The impact of relative intensity of exercise does not substantially affect the mood-enhancing qualities of exercise, but the greatest improvements in mood have been found when individuals are allowed to self-select their level of exercise intensity. The likely reason for this finding is the need to be realistically challenged by the task and the benefits of perceived control. When the demand

of the task (high intensity exercise) is perceived to exceed ability, anxiety and frustration are likely outcomes (Yeung, 1996). Therefore, the participants' expectations of performance are important to the mood-enhancing properties of an acute exercise bout.

Mood response to physical challenge in depression. Little is known about the mood response to acute physical challenge in depressed individuals. In one study, depression was associated with negative mood following exercise (Blackwood et al., 1998). Depressed patients showed increased negative mood to a bout of exercise (0.5 ± 1.0), compared to chronic fatigue syndrome patients (-0.1 ± 0.5; $p=0.05$) and control participants (-0.5 ± 0.76, $p=0.01$) (both comparison groups showed decreased negative mood to the bout of exercise). Furthermore, depressed patients have negative expectations about their performance which may lead to increases in negative mood (Kovacs & Beck, 1978). Therefore, it was hypothesized that an acute bout of exercise would not be mood enhancing for the depressed patients, but instead a mood decrement (see Hypothesis 1.B).

III. Cardiovascular Characteristics of Depression

a. Cross-Sectional Cardiovascular Correlates of Depression

Depression is associated with increased ambulatory blood pressure (Grewen et al., 2004). Depressed patients who are otherwise healthy often exhibit increased resting heart rates (HR) (Lahmeyer & Bellur, 1987) and reduced HR variability (Stein et al., 2000). The severity of depression is related to both resting heart rate and blood pressure, such that the higher the level of depressive symptoms, the higher the resting heart rate and blood pressure (Volkers et al., 2003; Volkers et al., 2004). These studies demonstrated an increase in both resting systolic (SBP) and diastolic blood pressures (DBP) as well as resting heart rate among depressed individuals (Carney et al., 1999; Moser et al., 1998). In the present investigation, resting cardiovascular measures (SBP, DBP, and HR) were assessed. These measurements were used to adjust for differences in cardiovascular measures at rest when investigating the cardiovascular reactivity to mental and physical challenge (see Methods section for more detail).

b. Cardiovascular Responsiveness to Mental and Physical Challenge in Depression

Light, Kothandapani, and Allen (1998) documented that the presence of subclinical depressive symptoms, as indicated by high scores on the Beck Depression Inventory, were associated with signs of enhanced peripheral sympathetic activity. The speech challenge task induced larger HR and cardiac output (CO) responses, along with a generalized decrease in HR variability and shortened PEP. These results indicate an increase in tonic sympathetic activity to the heart and an increase in sympathetically-mediated cardiovascular responsivity to behavioral stress (Light et al., 1998). Orthostatic challenge also induces increased HR changes in depressed participants compared to non-depressed controls, suggesting hyper-reactivity among depressed individuals (Carney et al., 1999).

In a recent meta-analysis of 21 studies, depression on cardiovascular reactivity to mental challenge were analyzed (21 involved SBP, 21 involved DBP, and 18 involved HR) (Kibler & Ma, 2004). The aggregate effect size for the relation between depressive symptoms and HR reactivity was moderate (d=0.37); aggregate effect sizes were smaller for SBP reactivity (d=0.13) and DBP reactivity (d=0.17). These findings provide support for the association between depressive symptoms and increased cardiovascular reactivity (see Hypothesis 2.A). However, there has been a lack of empirical studies investigating the acute effects of mental challenge in depressed patients on

mood and inflammation. The present investigation examined these parameters in depressed patients in conjunction with cardiovascular reactivity.

Depression can also affect cardiovascular responsiveness to an acute bout of exercise. A recent study suggests that MDD is associated with poorer exercise test performance, even after adjusting for important baseline variables including age, sex, use of beta-blockers, family history of coronary artery disease, and smoking status (Lavoie et al., 2004). Three indices of exercise test performance (patients' percentage of age-predicted maximum HR reached, peak exercise METS, and total exercise duration) were significantly lower in patients with MDD than in participants without MDD. These results suggest exercise performance is compromised in patients who are depressed. The MDD patients exhibited significantly greater SBP increases and a trend toward greater HR increases during exercise than patients without MDD (Lavoie et al., 2004). These findings provide support for the association between depressive symptoms and increased cardiovascular reactivity to acute exercise. However, there have been few empirical studies investigating the acute effects of physical challenge on cardiovascular parameters in depressed patients. The present investigation examined these parameters in depressed patients in conjunction with neurohormonal, emotional, and inflammatory reactivity. It was anticipated that elevated neurohormonal and increased negative emotional responses to exercise would be related to larger cardiovascular responses (see Hypothesis 2.B).

c. Inter-relation of Cardiovascular Correlates with Neurohormonal and Emotional Responsiveness

Sympathetic hyperactivation in response to both mental challenge and affective distress increases circulating levels of epinephrine and norepinephrine (Becker et al., 1996; Lampert et al., 2000). The augmentation of the sympathetic nervous system activity causes neurohormonal release and subsequent diverse effects on hemodynamic activity and vasomotor tone. The cardiovascular effects of mental stress are executed through cerebral neurochemical pathways associated with fear and anxiety. Many biological changes accompanying stress or emotional arousal are extended and intensified by a cascade of endocrine changes that help to modulate bodily response. These regulatory systems induce circulatory changes such as increases in blood flow to the muscles of the periphery, increases in heart rate and respiration, and otherwise prepare the organism for action and attention. Neurohormonal measures are also correlated with cardiovascular responses. Past research has demonstrated that in response to mental challenge, high HR reactors have significantly higher cortisol changes than low HR reactors (Sgoutas-Emch et al., 1994). Other investigations have revealed that heart rate reactivity is significantly correlated with ACTH ($r=0.50$; $p < 0.02$) (Lovallo et al., 1990) and cortisol changes ($r=0.62$, $p < 0.01$) (Sgoutas-Emch et al., 1994). In the present investigation, the correlation between neurohormonal changes and cardiovascular responses were examined in depressed individuals (see Hypothesis 2.B).

Emotional responses may mediate the relationship between neurohormones and CV reactivity (Gendolla & Krusken, 2001a). Moods are associated with catecholamine releases and other autonomic changes, including increases in HR, BP, body temperature, and skin conductance (Collet et al., 1997). It is possible that moods are able to influence cardiovascular adjustments through informational impact, such as appraisal of subjective demand (Forgas & Bower, 1987). Mental challenge tasks are therefore experienced as more difficult when they elicit negative mood as compared to positive mood. By means of this process, moods can impact the amount of effort mobilized, since the demand-effort relationship is proportional (Forgas & Bower, 1987). It follows that cardiovascular reactivity will be stronger in a negative than a positive mood condition (Gendolla & Krusken, 2001a). Persons in a negative mood state show stronger SBP reactivity during performance of a challenge task than persons in a positive mood (Gendolla & Krusken, 2001b). Therefore, mood valence (positive or negative) can influence the cardiovascular adjustments to a challenge task. The effect of mood state during challenge tasks on subsequent cardiovascular reactivity were examined in depressed patients in the current investigation (see Hypothesis 2.B).

IV. Immune System Parameters in Depression

a. Inflammatory Correlates of Depression

The inflammatory response can be triggered in a variety of ways, including infection and trauma. Inflammation is an important consequence of infection and injury and plays a primary role in the healing process. Proinflammatory cytokines including IL-1, IL-6, and tumor necrosis factor-α (TNF- α) attract immune cells to the site of infection or injury, and prime these immune cells to become activated to respond (Kiecolt-Glaser et al., 2002). Although the mechanisms associated with inflammation are critical to resolving infections and repairing tissue damage, chronic pro-inflammatory cytokine elevations can provoke pathological changes (Kiecolt-Glaser & Glaser, 2002).

Depression is associated with enhanced production of pro-inflammatory cytokines, including IL-6 (Dentino et al., 1999; Lutgendorf et al., 1999a; Maes et al., 1995; Maes et al., 1998). Importantly, both depressive symptoms and syndromal depression are associated with heightened plasma IL-6 levels (Lutgendorf et al., 1999a). In addition, successful pharmacologic treatment results in IL-6 level declines in patients with MDD (Sluzewska et al., 1995).

Overproduction of proinflammatory cytokines may lead to subsequent maladaptive immune and endocrine changes. IL-6 is a potent stimulator of CRH production, a mechanism that leads to heightened HPA activity, including elevated levels of plasma ACTH, followed by increased cortisol levels (Dentino et

al., 1999). Elevations in ACTH and cortisol can provoke multiple adverse immunological changes (Miller, 1998). Thus, there is a bi-directional association between inflammatory factors in neurohornonal parameters induced in the stress response (i.e., the HPA and SAM axes). As discussed in Section II, patients with MDD also have these increases in HPA and SAM axes activity.

b. Inflammatory Responsiveness in Depression

Both physical and psychological stress has been reported to increase plasma IL-6 concentrations (LeMay et al., 1990; Takaki et al., 1994; Zhou et al., 1993). Animal studies have shown that cytokines are secreted in response to stress in "depressed" rodents (LeMay et al., 1990; Shintani et al., 1995; Takaki et al., 1994; Zhou et al., 1993). With regard to the mechanism of stressor-induced IL-6 secretion, epinephrine administration to rats induces a rapid increase in plasma IL-6 concentrations, an effect that can be blocked by pretreatment with the β-adrenoceptor antagonist 1-propanolol (DeRijk et al., 1994). These observations suggest that stressor-induced increases in plasma IL-6 concentrations may be mediated by increased sympathetic activity and concomitant release of epinephrine from the adrenal medulla (Connor & Leonard, 1998).

Depression has also been associated with heightened platelet reactivity (Markovitz & Matthews, 1991; Steen & Holmsen, 1987). It is hypothesized that the increase in platelet reactivity is a consequence of increased sympathetic

output in response to various stressors (Lederbogen et al., 2004). Moreover, both physical and psychological stressors can provoke transient increases in proinflammatory cytokines (DeRijk et al., 1997; Song et al., 1999; Zhou et al., 1993). It is not known whether depression is associated with altered immune system responses to acute challenge such as mental and physical tasks. Therefore, the present study examined proinflammatory responses to mental and physical challenge tasks in depressed and non-depressed participants (see Hypothesis 3.A).

c. Inter-relation of Inflammatory Correlates with Neurohormonal and Emotional Responsiveness

The organism's adaptational response to various exogenous threats includes changes in the neuroendocrine-immune network (Bateman et al., 1989; Chrousos & Gold, 1992; Gold et al., 1988a; Gold et al., 1988b). The immune system and the neurohormonal output from both the HPA axis and the SAM are interconnected. One major component of the response to challenge is the HPA axis (as discussed in Section II). The other principal component of the response to challenge is the SAM (Gold et al., 1988a; Gold et al., 1988b). Both systems participate in a positive feedback loop so that activation of one system tends to activate the other system (Calogero et al., 1988; Valentino & Foote, 1988).

It is now well established that the HPA and SAM axes activity are also involved in the regulation of the immune response (Bateman et al., 1989;

Chrousos & Gold, 1992). The peripheral messengers of both systems are glucocorticoids and catecholamines. Both the messengers of the HPA and SAM axes have effects on the inflammatory response. Evidence exists that acute stress can cause an increase in inflammatory measures (Schulte et al., 1994). The HPA axis activates a number of immune system derived substances such as IL-1 and TNF-α (Berkenbosch et al., 1987; Sapolsky et al., 1987; Uehara et al., 1987). Therefore, neurohormones and inflammatory responses are mutually dependent processes.

Another consequence of challenge is the release of CRH. CRH induces lymphocyte proliferation (McGillis et al., 1989), increases expression of IL-2 receptor on T-lymphocytes (McGillis et al., 1989; Singh, 1989), and enhances the proliferative response of leukocytes (Singh, 1989). There are specific binding sites for CRH on leukocytes (Singh & Fudenberg, 1988). Normal volunteers injected intravenously with CRH showed a marked elevation of serum IL-1 and IL-2 levels as well as an increase in IL-2 receptor-positive lymphocytes despite elevated cortisol levels (Schulte et al., 1994). CRH (neurhormone) may be responsible for the effects of acute stress on the immune response.

Negative affect has been associated with immunological dysregulation in studies that have spanned the gamut from clinical depression and chronic stress to transient mood changes induced by laboratory manipulations (Herbert & Cohen, 1993). The effects of acute mental arousal on the immune system are complex (Ader et al., 1995). Cytokines (IL-1α and IL-2) are reported to increase with psychological challenge (Schulte et al., 1994), suggesting at least a partial

activation of the immune system (Kop & Cohen, 2001). Consistent with immune system activation is that acute phase proteins (CRP and fibrinogen) are also increased with psychological challenge (Dugue et al., 1993; Jern et al., 1989). Therefore, acute psychological challenges induce a partial activation of the immune system (Kop & Cohen, 2001).

Findings from a daily diary study demonstrated that antibody to an orally ingested antigen was higher in saliva on days when participants reported more positive moods and lower in saliva with more negative moods (Stone et al., 1993). Similarly, negative mood over the course of a day was associated with reduced NK cell lysis among women, and positive mood moderated this association (Valdimarsdottir et al., 2002). Among healthy older adults, decreased positive mood partially mediated the association between the stressor of moving and reduced NK cell lysis (Lutgendorf et al., 1999b). To document whether depression is characterized by exaggerated immune system responsiveness, over and above elevated baseline levels, the present study examined the effects of neurohormones and mood state on immune system reactivity in depressed individuals and non-depressed controls (see Hypothesis 3.B).

d. Interaction (bidirectionality) of cardiovascular and inflammatory responses to challenge

The magnitude of increases in inflammation and hemodynamic responses to mental stress are significantly correlated (Benschop et al., 1995). This suggests that dynamic, short-term changes in both systems are regulated by a common mechanism. It is hypothesized that SAM and/or HPA axis activity are potential common factors (Benschop et al., 1995; Benschop et al., 1998; Herbert et al., 1994; Zakowski et al., 1992). An increase in inflammatory markers in the peripheral blood is associated with an increase in epinephrine (Schedlowski et al., 1996). Epinephrine increases will also cause elevations of HR and BP. Several beta-adrenergic blockade studies have shown a simultaneous inhibition of stress-induced increases in inflammatory markers (e.g., IL-6) and cardiovascular measures (Bachen et al., 1995; Benschop et al., 1994; Benschop et al., 1996). Thus, the positive correlation between cardiovascular and inflammatory reactions to challenge may be caused by a common factor (sympathetic activation) that regulates both systems (Marsland et al., 1997). In the current project, cardiovascular and inflammatory reactions to mental and physical challenge were examined simultaneously to examine the interaction between these two systems.

V. Health Behavior Correlates of Depression

Depression is associated with a range of adverse health behaviors, such as cigarette smoking, low physical activity, and poor diet. These health behavior correlates of depression are interrelated phenomena (Woo, 2000) and important to consider as covariates in studies that examine cardiovascular and immune system related reactivity of depressed patients. Thus, the current project assessed these health behaviors as potential effect modifying or confounding factors.

a. Tobacco Smoking

Depression is associated with elevated prevalence of smoking and increased difficulties with tobacco smoking cessation (Glassman et al., 1990). The role of depression as a risk factor for smoking has been examined in several population-based samples. The first National Health and Nutrition Examination Survey used the Center for Epidemiological Studies Depression Scale (CES-D) to assess depression (Anda et al., 1990). These data provide evidence that the incidence of current smoking increases as the CES-D score increases (56% in the highest quintile of symptoms compared to 39% in the lowest quintile). Using the Diagnostic Interview Schedule (DIS), a structured interview designed for lay administration, the lifetime prevalence rate of smoking in an university-based smoking treatment clinic was approximately 40% for those having a history of

MDD compared to approximately 10% for those without a history of MDD (Hall et al., 1993). Although this figure is lower than reported previously (Glassman et al., 1990), it greatly exceeds the prevalence rate of MDD in the general population (approximately 10%).

Smoking stimulates the noradrenergic proteins in the locus coerulus which is similar to the effect of anti-depressant medications (Klimek et al., 2001). For that reason, it has been hypothesized that smoking may serve as a form of self-medication for individuals with depression (Klimek et al., 2001). It is also possible that smoking and depression are related by common sociological variables (e.g., low socioeconomic status).

Smoking can affect variables of interest in the present proposal (e.g., inflammatory markers and cardiovascular reactivity). For example, current smokers have increased C-reactive protein compared to non-smoking controls (Willerson & Ridker, 2004) and smoking is associated with elevated cardiovascular reactivity (Davis, 1999). The potential difference in smoking status between depressed and non-depressed participants was examined statistically in the present study.

b. Physical Activity

There is a growing literature on the psychological benefits of regular physical exercise to depression. Three basic types of exercise are: (i) cardiorespiratory or aerobic exercise (e.g., walking, jogging) in which oxygen is

metabolized to produce energy, (ii) muscular strength and isometric anaerobic exercise (e.g., weightlifting) in which energy is provided without the use of inspired oxygen; and (iii) flexibility exercise (e.g., yoga, stretching) that is designed to improve range of motion.

Cross-sectional studies of clinical and nonclinical samples have consistently found that active individuals report lower depression scores than sedentary individuals (Bhui & Fletcher, 2000; Hassmen et al., 2000; Ruuskanen & Ruoppila, 1995; Stephens, 1988; Weyerer, 1992). Large-scale prospective studies also suggest that regular physical activity is associated with lower scores on depression questionnaires at follow-up (Camacho et al., 1991; Farmer et al., 1988; Lampinen et al., 2000; Paffenbarger, Jr. et al., 1994). For example, in the Alameda County Study, Camacho and colleagues (1991) measured participants' activity levels and depressive symptoms in 1965, 1974, and 1983. Compared with men and women who reported higher activity levels, those who were inactive at baseline were at greater risk for higher depression scores at the first follow-up (OR=4.22 (3.17-5.62)). Participants who increased their physical activity between 1965 and 1974 were at no greater risk of depression in 1983 than those who were active throughout the period (OR=1.00 (0.63-1.59)). Conversely, those who became more inactive by 1974 were more likely to have depression scores in 1983 than those maintaining a high level of physical activity (OR=1.76 (1.06-2.92)).

Markers of low-grade inflammation are elevated in sedentary individuals (Mattusch et al., 2000; Rauramaa et al., 1986). Exercise interventions can

decrease the level of inflammatory markers. For example, in an investigation where marathon runners were followed for 9 months of training: median levels of C-reactive protein decreased from 1.19 mg/L to 0.82 mg/L, whereas a group of non-exercisers displayed no change during these same nine months (Mattusch et al., 2000). Therefore, regular physical activity may have a systematic effect on inflammatory markers. Engaging in regular physical activity is also associated with reduced cardiovascular reactivity to mental stressors (Spalding et al., 2004).

The potential differences in physical activity between depressed and non-depressed participants was examined statistically in the current investigation (Kohl et al., 1998).

c. Diet

Overweight and obesity are more common among depressed than non-depressed individuals (Stunkard et al., 2003). Associations between weight and depressive symptoms are not limited to major depression, but are also observed at lower levels of psychological distress (Istvan et al., 1992; Schlundt et al., 1990; Schlundt et al., 1990). Negative emotions are associated with lapses in dietary control (Cools et al., 1992; Greeno & Wing, 1994; Greeno & Wing, 1994). Between 34% to 54% of depressed patients gain weight during depressive episodes (DiPietro et al., 1992; Stunkard et al., 1991). Therefore, weight is an important behavioral factor that is different in depressed versus non-depressed individuals.

The relationship between increased markers of inflammation (e.g., CRP) and depression (see Section IV) is partially mediated by increased BMI (Miller et al., 2002). In fact, a recent study demonstrated a non-significant association between depressive symptoms and inflammation in normal weight participants, but the association was significant in obese participants (Ladwig et al., 2003). Also, overweight status is associated with increased cardiovascular reactivity (al Suwaidi et al., 2001). Because weight affects the relationship between depression and the biological measures used in this study, the depressed patients and non-depressed controls were matched on body-mass index.

Rationale and Hypotheses

The research reviewed in the background sections indicates that depression is associated with various emotional and neurohormonal changes that may adversely affect cardiovascular and inflammatory parameters. The increases in negative emotions and neurohormones to both mental and physical challenges are associated with increases in cardiovascular and inflammatory reactions in healthy non-depressed individuals, but little is known about these associations in patients with MDD. These elevated responses of physiological and biological parameters may partially explain the increased morbidity and mortality in depressed individuals compared to their non-depressed counterparts.

The hypotheses of the proposed investigation are based on the model presented in Figure 1. It is expected that individuals with depression will have

exaggerated responses in neurohormones and negative mood to both mental and physical challenge (regardless of the elevated baseline levels among depressed individuals versus controls) (Hypothesis 1). Because of the limited research on biobehavioral reactivity in depression, no specific hypotheses were formulated to address differences in reactivity between mental versus physical challenges. This study further examines whether the elevated responses of neurohormones and negative mood to mental and physical challenge tasks lead to increased cardiovascular (Hypothesis 2) and inflammatory (Hypothesis 3) responses. The relationship between elevated neurhormonal and mood responses leading to increased cardiovascular and inflammatory reactions are expected to be present in depressed individuals as well as non-depressed controls because these relationships are inherent to the stress response. In other words, this investigation postulates a mediational model, such that individuals with depression have increased cardiovascular and inflammatory responses because of the hyper-reactivity of neurohormones and negative mood to mental and physical challenge tasks.

The cardiovascular and inflammatory outcomes in this study are both products of the HPA and SAM axes. Therefore, consistent with prior studies, a correlation between cardiovascular and inflammatory reactions is anticipated. Because these outcomes may reflect the same underlying stress response, the direction of this relationship (i.e., whether cardiovascular reactions cause the inflammatory reactions or vice-versa) will not be examined in this study.

Specifically, to study the effect of mental and physical challenge in depressed individuals, the following hypotheses will be examined:

Hypothesis 1

A) Individuals with depression will have increased neurohormonal responses (ACTH, cortisol, norepinephrine, and epinephrine) to both mental and physical challenge as compared to non-depressed controls.

B) Individuals with depression will have increased negative mood responses to both mental and physical challenge as compared to non-depressed controls.

C) The magnitude of increase in negative mood responses to both mental and physical challenge will be related to the magnitude of increases in neurohormonal responses during these challenge tasks.

Hypothesis 2

A) Individuals with depression will have higher cardiovascular reactivity (SBP, DBP, and HR) during both the mental and physical challenge tasks as compared to non-depressed controls.

B) The cardiovascular increases during both mental and physical challenge tasks will be related to the neurohormonal and negative mood responses during these challenge tasks.

Hypothesis 3

A) Individuals with depression will have increased markers of inflammation (IL-6, CRP, and TNF-α) in response to both mental and physical challenge as compared to non-depressed controls.

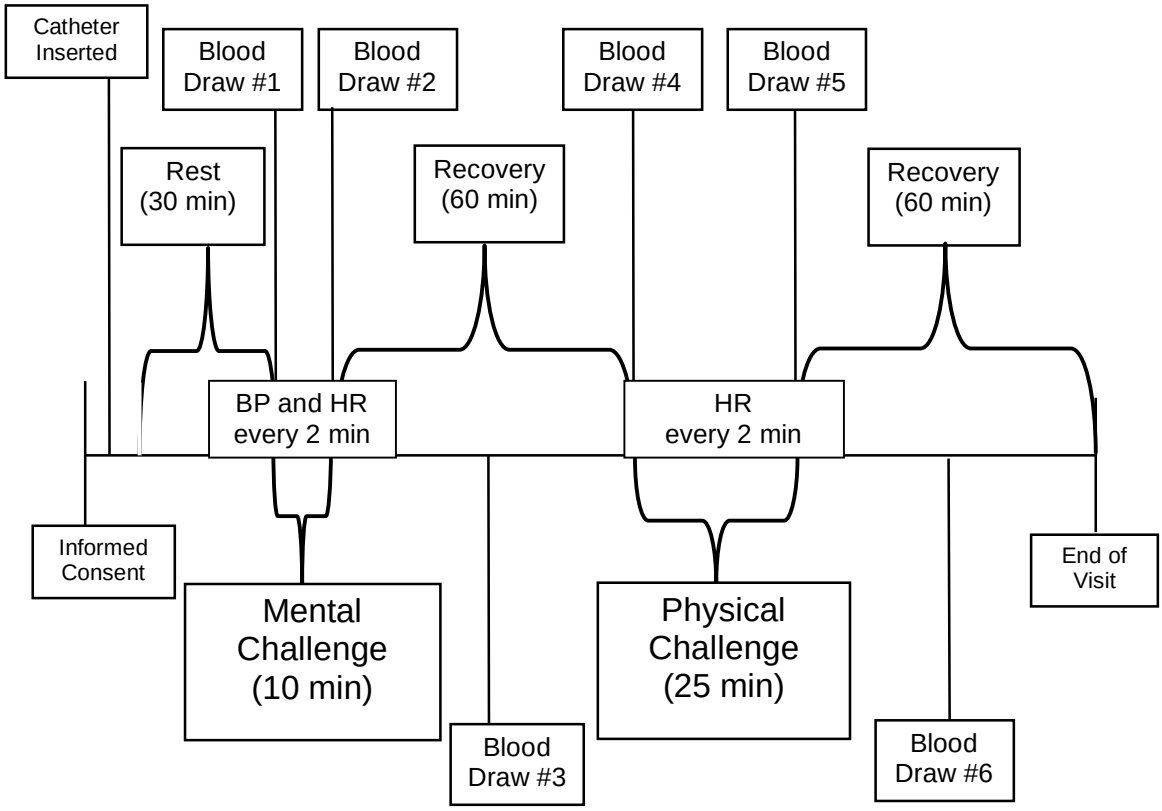
B) The inflammatory increases during both mental and physical challenge tasks will be related to the neurohormonal and negative mood responses during these challenge tasks.

Research Design and Methods

I. General Overview

Psychological and physiological parameters were assessed in 14 participants with depression and 16 non-depressed controls (Total N = 30). As shown in Figure 3, participants were tested in one continuous laboratory visit. All patient testing and data collection was performed at Uniformed Services University of the Health Sciences. Biochemical analyses were performed at the University of Vermont (Dr. Russell P. Tracy) and Emory University (Dr. Robert Bonsall). The University of Vermont analyzed IL-6, TNF-α, and C-reactive protein and Emory University analyzed norepinephrine, epinephrine, adrenocorticotropin hormone, and cortisol (coefficients of variability presented in Section IV.e). These laboratories have used these assays for a wide range of NIH funded studies.

Figure 3.



II. Participants

Depressed Participants: Fourteen individuals with depression participated in this study. The current project defined depression status by the use of a standardized questionnaire. Inclusion criteria was: (1) Depression as defined by the Beck Depression Inventory-II (BDI-II) as scores 10 and greater (Beck et al., 1996). Exclusion criteria were: (1) Age < 18 or >80; (2) History of coronary artery disease (myocardial infarction, cardiovascular revascularization procedures, and/or chest pain on exertion); (3) Use of anti-hypertensive medication; (4) Use of immunomodulatory or anti-inflammatory medications other than aspirin; (5)

History of bipolar disorder or treated for psychosis; (6) Current treatment by a psychiatrist or psychologist for a mental disorder other than depression and anxiety; (7) Active suicidal ideation as determined by a positive response to question #9 on the BDI-II. Suicidal ideation reported by participants would have lead to exclusion from the research project and immediate referral to a mental health professional; (8) Refusal of informed consent.

All depressed participants were either under psychiatric and/or psychological treatment for depression or were provided a referral if current depression is not being treated. All structured interviews were conducted by a licensed clinical psychologist (Dr. Jennifer Francis). She also assisted in finding appropriate referrals for participants who were not being treated for their depression at study enrollment.

Healthy controls: Sixteen individuals without depression as defined by the BDI-II (scores < 10) participated in this study. The healthy controls were matched to the depressed participants on gender, age, and BMI (see Section IV.f. for rationale). Inclusion criteria: (1) Beck Depression Inventory-II (BDI-II) with scores less than 10 (Beck et al., 1996). Exclusion criteria were: (1) Age < 18 or > 80; (2) History of coronary artery disease (myocardial infarction, cardiovascular procedures, and/or chest pain on exertion); (3) Use of anti-hypertensive medication; (4) Use of immunomodulatory or anti-inflammatory medications other than aspirin; (5) History of or current treatment for a psychiatric or psychological disorder; (6) Refusal of informed consent.

III. Procedures

Participant testing and data collection were performed at the Uniformed Services University of the Health Sciences (USUHS, Bethesda, MD) in the Human Performance Laboratory (supervised by Dr. Patricia Deuster). After participants provided written informed consent, the BDI-II questionnaire was administered by a trained researcher to determine depression status. Questionnaires were then completed to evaluate demographic information. The following information was obtained: age, race/ethnicity, height, weight, current medication, and exercise level (Kohl et al., 1998). After placement of an indwelling catheter and a blood pressure monitor (see Section IV for details), a 30-minute baseline period was taken to establish baseline hemodynamic measures in a resting condition. Two challenge tasks were then used to evaluate mental challenge responses (anger recall/mental arithmetic) and physical challenge (exercise) responses. Following mental stress, a 60-minute recovery period was observed after which participants engaged in a submaximal exercise test (Figure 3).

It was important to include both a mental and a physical challenge task in order to thoroughly investigate the possible reactivity changes induced by depression status and whether these reflect altered responsiveness to psychological challenge per se, or exaggerated reactivity in general. Details of these tasks are described below.

IV. Measures Obtained During the Study

a. Assessment of Depressive Symptoms

Standardized measures were used to assess depressive symptoms and are presented in Appendix A. Depression was assessed using the Beck Depression Inventory-II (BDI-II) (Beck et al., 1996) as the primary diagnostic tool. This inventory consists of 21 items and has demonstrated excellent reliability (Cronbach's $\alpha = 0.92\text{-}0.93$). BDI-II scores exceeding 10 are considered indicative of the potential presence of depression (Beck et al., 1996).

The Hamilton Rating Scale for Depression (HRSD) (Hamilton, 1960; Hamilton, 1967) was also used to assess depression. The advantage of the HRSD over other structured interviews is that it enables evaluation of the *severity* of depression. The HRSD is a 21-item interview assessing emotional, behavioral and somatic symptoms of depression. Overall inter-rater agreement is high, commonly exceeding 0.84 (Hedlund & Virmani, 1979). Total scores range from 0 to 52, with scores from 7-17 reflecting mild depression and scores above 17 indicating clinical depression (Hamilton, 1960; Hamilton, 1967).

In addition, a licensed clinical psychologist confirmed Major Depressive Disorder diagnosis by conducting the Structured Clinical Interview for DSM-IV (SCID). The SCID is generally regarded as a standard in assessing psychiatric disorders (First et al., 1995). It has been utilized as the criterion standard for assessing the presence of depressive disorders (Lowe et al., 2004).

b. Mental and Physical Challenge Testing

Participants were tested between 12 and 4 pm to control for the effects of time of day on biological and behavioral parameters. After placement of the indwelling catheter and blood pressure monitor, a 30-minute resting period was taken to determine baseline hemodynamic and neurohormonal measures. Then, participants performed an anger recall task. This task involved a speech about a recent incident that elicited a frustration or angry response (Ironson et al., 1992; Kop et al., 2004). The anger recall task induced significant hemodynamic responses and allowed assessment of emotional responses to personally relevant situations.

Immediately following the anger recall task, a five-minute mental arithmetic task was performed. In the mental arithmetic task, participants were asked to subtract serial sevens from a four-digit number while being encouraged to work as fast and accurately as possible (Gottdiener et al., 1994; Kop et al., 2004). The mental arithmetic task elicits a hemodynamic, hemostatic, and ischemic responses (Blumenthal et al., 1995; Jern et al., 1991; Kop et al., 1998; Krantz et al., 1996). Previous research has demonstrated that the effects induced by this mental challenge task are not an artifact of talking per se (Rozanski et al., 1988).

After completion of the anger recall and mental arithmetic tasks, a 60-minute resting period was applied and repeated hemodynamic measurements

performed to determine return to baseline of cardiovascular parameters. The order of the mental challenge tasks was counter-balanced.

The rationale for the consecutive performance of anger recall and mental arithmetic was based on the fact that some immune system parameters require stress exposures of greater than five minutes to display a detectable response (Kunz-Ebrecht et al., 2003).

The physical challenge task consisted of a gradual treadmill exercise test with a 5-minute warm-up period. All participants performed the warm-up period at 3.0 mph with a grade of 2.0. The HR response to the warm-up period determined the speed of the subsequent stages (Table 1). The two subsequent stages were 10 min at approximately 70% of VO_{2max} (grade of 10.0) and 5 min at approximately 90% of VO_{2max} (grade of 10.0) (see Appendix B for rationale of exercise protocol).

Table 1. Physical Challenge Speed Protocol

Warm-Up HR	Stage 1 Speed	Stage 2 Speed
< 100	4.0	4.6
100-119	3.4	4.0
>119	2.8	3.4

c. Hemodynamic Measures

During baseline and mental challenge, blood pressure (BP) and heart rate (HR) was obtained at 90 second intervals using a Critikon Dinamap automated cuff placed on the arm. This procedure enabled determination of increases in hemodynamic measures (systolic and diastolic BP and HR) while adjusting for baseline levels. Baseline hemodynamics were determined by averaging the last three measures during the 30-minute rest period. During the mental challenge tasks hemodynamics were assessed at 30 seconds, 2 min., and 3 min. 30 sec. into each of the two tasks and the peak value per task was used in analyses. During the physical challenge, heart rate was assessed at the end of each stage and during recovery (at 2 min. intervals) until values returned to baseline (± 5 beats per minute).

Three general strategies for calculating reactivity scores have been described: (1) the aggregated baseline change scores strategy where all the baseline measures prior to a series of tasks are averaged to compute an overall baseline, that is subsequently subtracted from individual task reactivity; (2) the residual change score method, where a regression line is calculated for the relationship between baseline and task measures and then the residual values from the regression line are used as the reactivity measures; and (3) the arithmetic change scores, calculated by subtracting the peak task measures from the preceding baseline measures (Kamarck et al., 1992; Manuck et al., 1989).

Kamarck et al. (1992) support an aggregated baseline across tasks as the proper manner to perform a baseline cardiovascular evaluation because the "baseline" cardiovascular measures tend to drift upwards across repeated challenge tasks. However, in the Kamarck et al. (1992) study there were three challenge tasks, each lasting for approximately 6-10 minutes with a minimal resting period (< 5 min) between each task (Kamarck et al., 1992). Therefore, the challenge period was quite long and the recovery time relatively short. In the present study, a longer recovery period of 30 minutes will be used between mental challenge and physical challenge. Because the recovery periods in the present study are longer than in the Kamarck et al. (1992) protocol, baseline drift is not expected to occur in the present investigation.

Manuck et al. (1989) provide evidence in support of a residual change score approach. The residualized change score provides a means of quantifying the physiologic responses to challenge tasks, while adjusting for the influence of baseline levels on these responses. Although, there are occasions in which the residualized change scores differ from the basic arithmetic change scores, these occasions are rare (Manuck et al., 1989). The reliability of both residualized change scores and arithmetic change scores are comparable (Kamarck et al., 1992). Furthermore, the residual change score approach assumes a linear relationship between baseline and task levels, which is not necessarily true in all circumstances.

Therefore, arithmetic change scores from baseline to peak levels during the mental and physical challenge tasks were used in this study because this

method is directly based on the raw data and it is comparable in reliability and outcome to the other two methods (Kamarck et al., 1992; Manuck et al., 1989). Although it is important to note that the strategy chosen does not control for baseline values.

d. Emotional Reactivity Measures

Prior to and at the completion of each challenge task (anger recall, mental arithmetic, and exercise), participants completed emotional ratings to determine both the baseline and task emotions. Three subscales of the short-form POMS (POMS-SF) were used for these assessments (see Appendix C). The POMS-SF consists of the same six subscales of the original POMS (anger, fatigue, confusion, tension, and vigor). The original scale contains 65 items, whereas the short-form contains 30 items. The anger, fatigue, and vigor subscales of the POMS-SF (15 items) were used to keep the participant burden to a minimum. These are the most relevant subscales to the challenge tasks. Each participant was asked to rate the level of mood on a 7-point Likert scale from “not at all” to “extremely.” The Cronbachs’ α’s range from 0.85 to 0.97 on the three subscales (McNair et al., 1992). Also, the POMS-SF correlates with the full version of the POMS (r=0.95) (McNair et al., 1992). Six additional emotions were added to the three scales of the POMS-SF to ensure coverage of different emotional domains. Interested, challenged, anxious, depressed, irritated, and frustrated were assessed with 7-point Likert scales that range from “not at all” to “extremely.”

Attributions were assessed by the same 7-point Likert scales assessing feelings of control and failure (23 total Likert items will be used) (see Appendix C). The arithmetic change score strategy was used to determine emotional reactivity.

e. Blood Sampling and Storage

A 19-gauge catheter was used and participants rested for 30 minutes prior to the first (baseline) blood draw of 30 mL. The first 5 mL blood was be discarded. A trained technician at HPL conducted the blood draws. Blood samples were collected in vacuum tubes containing dry reagents and mixed by gently inverting the tube for 30 seconds; EDTA (4.5 mmol/L) samples were kept at 4^{o} C and separation of plasma was performed by centrifugation at 3000g for 15 minutes. For measurement of catecholamines, plasma was decanted into storage tubes containing 1.25 µmoles sodium EDTA and 1.25 µmoles sodium metabisulfate to inhibit oxidation. Aliquots of plasma were then stored at -70 ^{o} C until analysis.

Neurohormonal Measures. The neurohormonal analyses were conducted at Emory University (Dr. Bonsall).

Cortisol: Cortisol was assayed in duplicate 10 µl aliquots of EDTA plasma by a solid phase radioimmunoassay (RIA) using materials obtained from DiaSorin Corporation (Stillwater, MN: sensitivity 0.2 µg/dl (2 ng/mL); inter- and intra-assay CV <4%. Use of this assay has been described previously (Becker et

al., 1996; Goldberg et al., 1996; Kaufmann et al., 1998). Standards (range 1 to 30 ng/mL) consist of serum standards diluted with 200 µl of phosphate-buffered saline. Protein concentrations are equalized in standards and samples by adding cortisol-free serum to the samples. Sensitivity is 0.01 µg/dL (0.1 ng/mL) and inter- and intra-assay CV <6%.

ACTH: ACTH was assayed in duplicate 200-µl aliquots of EDTA plasma by a two-site immunoradiometric method using materials obtained from the Nichols Institute (San Juan Capistrano, CA). This procedure is highly specific and exhibits limited cross-reactivity with related physiologic peptides. Sensitivity of the assay is 1 pg/mL (0.22 pmole/L) with inter- and intra-assay CV <6%. Nichols announced potential discontinuation of their ACTH IRMA. An alternative supplier (DiaSorin) has been evaluated and was a good substitute for the Nichols assay (Raff & Findling, 1989).

Catecholamines: Epinephrine and norepinephrine were measured in plasma following alumina extraction and on-line cation enrichment (Becker et al., 1996; Goldberg et al., 1996; Kaufmann et al., 1998; Kilts et al., 1984). Duplicate sample aliquots (0.25 – 1.0 ml) were taken to mildly basic pH with Tris and extracted with acid-washed, screened alumina. Catecholamines were desorbed from the alumina using 0.1 M perchloric acid, and extracts are analyzed by reverse-phase, ion-pair high performance liquid chromatography in combination with a computer-controlled cation-enrichment pre-column and a 3-electrode electrochemical detector connected to a computerized data-acquisition system. NE intra-assay CVs are 6.6% (<400 pg/ml), 6.5% (400 to 800 pg/ml) and 7.1%

(>800 pg/ml), mean inter-assay CV for pooled samples in the range 300 to 550 pg/mL =10.3%, and blanks read 6.0 ± 10.3 (S.D.) pg/mL. For epinephrine, mean intra-assay CV are 27.1% (<40 pg/mL), 13.5% (40 to 80 pg/mL) and 9.6% (>80 pg/mL); mean inter-assay CV for pooled samples in the range 60 to 140 pg/mL is 16.3%, and blanks read 7.0 ± 14.5 (S.D.) pg/mL.

Immune system measures. The immune system parameters were selected based on prior clinical, epidemiological, and experimental investigations (see Background section) and were conducted at the University of Vermont (Dr. Tracy).

Cytokines: Plasma IL-6 and TNF-α were measured by an enzyme-linked immunosorbent assay (ELISA) (Quantikine HS Human IL-6 Immunoassay; R&D Systems, Minneapolis, MN). The detection range is 0.156-10.0 pg/mL. Prior intra- and interassay coefficients of variance were 5.6% and 8.6%, respectively and the dynamic range of this high sensitivity assay was 0.256 to 10 pg/mL. All samples from a participant subject were analyzed in one assay to eliminate interassay variations. The laboratory CVs for these assays are approximately 6.3%.

Acute Phase Protein: C-reactive protein (CRP) was measured using the BNII nephelometer from Dade Behring using a particle enhanced immunonepholometric assay. Polystyrene particles are coated with monoclonal antibodies to CRP that agglutinate, causing an increase in the intensity of scattered light, in the presence of CRP. The increase in scattered light is proportional to the amount of CRP in the sample. The assay range is 0.175–1100

mg/L. Expected values for CRP in normal, healthy individuals are <3 mg/L. Intra-assay and inter-assay CVs range from 2.3–4.4% and 2.1–5.7%, respectively (Harris et al., 1999; Macy et al., 1997).

f. Assessment of Effect Modifying Variables and Health Behaviors

Depression is associated with a range of adverse health behaviors affecting the immune system, such as tobacco smoking, low physical activity, and poor diet (see Background Section V). Because smoking may be an effect-modifying factor, self-reported smoking status was investigated statistically as a possible confounding variable. Women are twice as likely as men to experience an episode of depression (in child-bearing years) (Sloan & Kornstein, 2003). Gender is also associated with different symptom reports, quality of life, inflammation markers, and neurohormonal measures (Moore, 1995; Gottlieb et al., 2004). Therefore, the groups were also matched on gender. Depressive symptomatology, inflammatory markers, and fitness all are changed by the aging process (Chodzko-Zajko & Ringel, 1987; Krause & Clark, 2001; Kurachi et al., 2000; Snowdon, 2001). The groups were matched on age using a 5-year matching range per case. Statistical methods were used to adjust for possible confounding by low physical activity as assessed by a standardized questionnaire (Kohl et al., 1998).

Statistical Analyses and Power Calculation

Previous findings were used to estimate effect sizes for the current investigation. The proposed sample size of 50 participants is sufficient to examine all hypotheses at a power >80% (β<0.20) with a Type I error (α) set at <0.05 (two-tailed). To test the hypotheses of the current proposal, we addressed the statistical analysis and power for each of the hypotheses separately. All power analyses were performed with the nQuery Advisory power calculation software package.

Hypothesis 1a: Individuals with depression will have increased neurohormonal responses (ACTH, cortisol, norepinephrine, and epinephrine) to both mental and physical challenge as compared to non-depressed controls.

Statistical analysis: To examine the association between depression and the increased neurohormonal response during the challenge tasks, a series of between subjects t-tests were used, where the depressed group was compared to the non-depressed group. The dependent variables were ACTH, cortisol, norepinephrine, and epinephrine. The biochemical data displayed a non-normal distribution, therefore a natural log-transformation was applied prior to analyses (Hoaglin et al., 1983). Data were presented in tables as geometric means. Specifically, means and 95% confidence intervals will be presented on the natural log transformed data. Then the results were exponentiated to present the data on the original scale. In addition, repeated measures analysis of variance

(ANOVA) were used to examine the interaction between group status (depressed vs. non-depressed) and responses from baseline.

Power computation: Based on prior research, we expected the effect size to be of the order of a one standard deviation difference (Deuster et al., 2000). Power analyses were based on the between subjects' t-tests for group differences. Using the procedures of power estimation (Cohen, 1988), this effect size required 34 participants (17 in each group) to detect between-group differences with p=0.05 and a power of 0.80.

Hypothesis 1b: Individuals with depression will have increased negative mood responses to both mental and physical challenge as compared to non-depressed controls.

Statistical analysis: To examine the association between depression and the increased negative mood during the challenge tasks, we used a series of between-groups t-tests, where the depressed group was compared to the non-depressed group. The dependent variables were the arithmetic changes scores of the fatigue, anger, and vigor scales of the POMS-SF, along with the additional Likert items. Mixed-model repeated measures ANOVA were also conducted parallel to the models used in Hypothesis 1a.

Power computation: Based on prior research, we expected the effect size to be of the order of a two-thirds standard deviation difference (Feldman et al., 1999). Power analyses are based on the proposed between subjects' t-tests for group differences. Using the procedures of power estimation (Cohen, 1988), this effect

size required 37 participants (approximately 18 in each group) to detect between-group differences with p=0.05 and a power of 0.80.

Hypothesis 1c: The magnitude of increase in negative mood responses to both mental and physical challenge will be related to the magnitude of increases in neurohormonal responses during these challenge tasks.

Statistical analysis: To examine the association between negative mood responses and neurohormonal responses to the challenge tasks, we used a series of correlations, where negative mood responses were correlated with neurohormonal responses, using arithmetic change scores. The biochemical data displayed a non-normal distribution, therefore log-transformation was applied prior to analyses (Hoaglin et al., 1983). When correlations were statistically significant, regression analyses were performed to determine if the correlation was statistically significant after controlling for group status.

Power computation: Based on prior research, we expected the correlations (r) to be in the order of 0.40 (Grewen et al., 2004). Forty-seven participants were needed to detect a correlation of r=0.40 with p=0.05 and a power of 0.80.

Hypothesis 2a: Individuals with depression will have higher cardiovascular reactivity (SBP, DBP, and HR) during both the mental and physical challenge tasks as compared to non-depressed controls.

Statistical analysis: To examine the association between depression and the increased cardiovascular response during the challenge tasks, we used a series of between-groups t-tests, where the depressed group was compared to the non-depressed group. The dependent variables were HR, SBP, and DBP. As in

Hypothesis 1a analysis, we also used mixed-model ANOVA to examine group differences in reactivity.

Power computation: Based on prior research, we expected the effect size to be of the order of one standard deviation difference (Kibler & Ma, 2004). This effect size required 34 participants (17 in each group) to detect between-group differences with p=0.05 and a power of 0.80.

Hypothesis 2b: The cardiovascular increases during both mental and physical challenge tasks will be related to the neurohormonal and negative mood responses during these challenge tasks.

Statistical analysis: To examine the association between cardiovascular responses and mood/neurohormonal responses to the challenge tasks, we used a series of correlations, where cardiovascular responses were correlated with the mood/neurohormonal responses, using arithmetic change scores as variables in the model. If these correlations were statistically significant, multivariate regression analyses were performed to determine if the correlations were statistically significant after controlling for group status.

Power computation: Based on prior research, we expected the correlations to be in the order of 0.50 (Lovallo et al., 1990). This required 30 participants to detect a correlation of 0.50 with p=0.05 and a power of 0.80.

Hypothesis 3a: Individuals with depression will have increased markers of inflammation (IL-6, TNF-α, and CRP) in response to both mental and physical challenge as compared to non-depressed controls.

Statistical analysis: Analyses were parallel to those for Hypothesis 1a. To examine the association between depression and increased inflammatory responses during the challenge tasks, a series of between-groups t-tests were examined, where the depressed group was compared to the non-depressed group. The dependent variables were IL-6, TNF-α, and C-reactive protein. The biochemical data displayed a non-normal distribution, therefore natural log-transformation was applied prior to analyses (Hoaglin et al., 1983). Data were presented in tables as geometric means, as described above.

Power computation: Based on prior research, we expected the effect size to be of the order of one standard deviation difference (Takaki et al., 1994). This effect size required 34 participants (17 in each group) to detect between-group differences with p=0.05 and a power of 0.80. Mixed-model ANOVAs were also conducted, with the interaction term used to examine altered reactivity among depressed individuals.

Hypothesis 3b: The inflammatory increases during both mental and physical challenge tasks will be related to the neurohormonal and negative mood responses during these challenge tasks.

Statistical analysis: Analyses were consistent with the models used to examine Hypothesis 2b. To examine the association between inflammatory responses and mood and neurohormonal responses to the challenge tasks, we used a series of correlations, where inflammatory responses were correlated with the mood and neurohormonal responses. The biochemical data appeared to display a non-normal distribution, natural log-transformation were therefore applied prior

to analyses (Hoaglin et al., 1983). Regression analyses were conducted parallel to those used in Hypothesis 2b.

Power computation: Based on prior research, we expected the correlations to be in the order of 0.40 (Stone et al., 1993). This required 47 participants to detect a correlation of r=0.40 with p=0.05 and a power of 0.80.

Results

I. Participant Characteristics

Analyses included data from 30 participants (15 males and 15 females): 14 depressed and 16 controls. As shown in Table 2, the groups did not differ on race, marital status, years of education, income, job status, smoking status, living arrangements, and physical activity level (ρ values >0.20). Matching for age, gender, and BMI was successfully accomplished (Table 2). Only two of the depressed participants were using selective serotonin reuptake inhibitors, therefore no subgroup analyses were conducted comparing participants with versus without anti-depressive pharmacotherapy (Table 2).

In addition, no significant differences existed between depressed and control participants at baseline on SBP, t(28) = 7.52, ρ = 0.68 (depressed: *M* = 111.5 mmHg, *SD* = 23.7; control: *M* = 108.7 mmHg, *SD* = 10.6); DBP, t(28) = 5.0, ρ = 0.12 (depressed: *M* = 73.3 mmHg, *SD* = 12.2; control: *M* = 67.7 mmHg, *SD* = 4.7); and HR, t(28) = 0.05, ρ = 0.81 (depressed: *M* = 67.7 beats/minute, *SD* =

6.1; control: *M* = 67.2 beats/minute, *SD* = 5.8). No significant differences were found between depressed and control female participants on current menstrual cycle phase. Specifically, the groups were at comparable days since their last menstrual cycle ($t(13) = 0.48$, $\rho = 0.78$ (depressed: *M* = 12.1 days, *SD* = 6.6; control: *M* = 13.4 days, *SD* = 9.3)). Menstrual cycle was investigated further by breaking the cycle into the follicular (days 1-14) and luteal (days 15 or later) phases. For the control participants, 50% were in the follicular phase and 50% were in the luteal phase. In the depressed group, 57% were in the follicular phase and 43% were in the luteal phase. Using a Chi-Square analysis, this difference in categories was not statistically significant ($\chi^2 = 0.08$, $\rho = 0.78$). Gender was examined for differing emotional, neurohormonal, cardiovascular, and inflammatory responses to mental and physical challenge among males and females, but no statistically significant differences were observed.

II. Assessment of Depressive Symptoms

Depressive symptoms were assessed using the BDI-II and the HRSD. The depressed participants were required to score at least 10 on the BDI-II to be eligible for the study and the control participants were required to score less than 10. In light of these requirements, a difference existed between the depressed and control participants on the BDI-II (depressed: *M* = 27.1, *SD* = 12.0; control: *M* = 1.6, *SD* = 2.4). The difference in depressive symptoms was also observed when examining the HRSD (depressed: *M* = 17.1, *SD* = 6.6; control: *M* = 1.1, *SD*

= 1.1). Statistical tests were not conducted for these comparisons because depression status was a selection criterion for this study, thus a priori identifying the two groups to be derived from different populations.

A licensed clinical psychologist performed the SCID to confirm Major Depressive Disorder diagnosis for the depressed participants. Twelve of the 14 depressed participants qualified for full MDD diagnoses. The other two participants were diagnosed with Minor Depression. All analyses were performed with the total depressed group (n=14). Secondary analyses were used to examine the sub-group with SCID diagnosed MDD (n=12), and results did not differ from the entire sample with depression (n=14). Therefore, all analyses presented involve the total depressed group of 14 participants. One participant in the depressed group qualified for an atypical MDD-specifier diagnosis, the other 13 participants endorsed typical symptoms, therefore no analyses were conducted comparing typical versus atypical depressed participants.

III. Hypothesis 1A: Neurohormonal responsiveness to challenge as related to depression

a. Neurohormonal responses to mental challenge

Participants in the depressed group did not differ from participants in the control group on baseline measures of ACTH, cortisol, NE, and Epi (ρ's > 0.10). As discussed in the Methods section, reactivity to challenge (both mental and physical tasks) was investigated using arithmetic change scores. In response to

the mental challenge, the depressed participants showed an immediate increased reactivity of ACTH ($t(22) = 2.35$; $\rho = 0.03$). Thirty minutes after completion of mental challenge, the depressed group displayed increased cortisol reactivity ($t(23) = 4.13$; $\rho = 0.001$). ACTH thirty minutes after mental challenge and cortisol immediately after the mental challenge did not demonstrate different reactivity patterns between the groups (ρ's > 0.85) (Table 3).

The control group did not significantly respond to the mental challenge based on ACTH, cortisol, or Epi, whereas there was a significant effect of mental challenge on the control participants' NE levels (Table 3). The depressed participants showed increased reactivity on NE ($t(23) = 2.15$; $\rho = 0.04$) and Epi ($t(23) = 2.16$; $\rho = 0.04$) compared to the control participants (Table 3).

b. Neurohormonal responses to physical challenge

All of the participants performed the same exercise warm-up period. Based on the warm-up HR, the speed and grade of the treadmill was set. Therefore, the participants performed different exercise protocols based on their response to the warm-up period, as discussed in the methods section. Three different protocols were utilized (see Appendix B). In the depressed group, 6 participants (43%) were in the fastest protocol, 6 participants (43%) were in the middle protocol, and 2 participants (14%) were in the slowest protocol. In the control group, 8 participants (53%) were in the fastest protocol and 7 participants (47%) in the middle protocol. Using a Chi-Square analysis, this difference in

exercise protocol assignment based on warm-up heart rate was not statistically significant between depressed and control participants ($\chi^2 = 2.33$, $\rho = 0.31$).

In response to the physical challenge, the control participants showed increased reactivity of ACTH at the peak of exercise ($t(10) = 1.96$; $\rho = 0.01$). ACTH during warm-up, ACTH thirty minutes after the physical challenge, cortisol during warm-up, cortisol at the peak of exercise, and cortisol thirty minutes after the physical challenge did not show different reactivity patterns between the groups (ρ's > 0.30) (Table 4).

In response to the physical challenge, the groups did not demonstrate different reactivity patterns in NE and Epi during warm-up or peak exercise (ρ's > 0.40) (Table 4).

IV. Hypothesis 1B: Negative mood responses to challenge as related to depression

a. Negative mood responses to mental challenge

Measures of negative mood included the fatigue, vigor, and anger subscales from the POMS-SF and individual 7-point Likert scales (depressed, interested, challenged, anxious, irritated, frustrated, feeling like a failure, and in control) were used to assess different emotional domains and attributions.

At baseline, differences existed between the depressed and control participants on anger, fatigue, depression, challenge, anxiousness, frustration,

irritation, and feeling like a failure (ρ's < 0.05, Table 5). No significant differences were observed in interest, control, or vigor (ρ's > 0.05, Table 5).

In response to the mental challenge, the depressed participants showed increased reactivity on the following negative mood measures: fatigue ($t(28) = 2.24$; $\rho = 0.04$) and depressed ($t(28) = 2.86$; $\rho = 0.01$). The control participants had increased reactivity on feeling anxious ($t(28) = 2.41$, $\rho = 0.02$) and challenged ($t(28) = 3.18$; $\rho < 0.01$). No significant differences for mood reactivity were found on the following negative mood measures: anger ($t(28) = 0.61$; $\rho = 0.55$), vigor ($t(28) = 0.17$; $\rho = 0.87$), interested ($t(28) = 0.44$; $\rho = 0.66$), irritated ($t(28) = 0.35$; $\rho = 0.73$), in control ($t(28) = 1.66$; $\rho = 0.11$), like a failure ($t(28) = 1.95$; $\rho = 0.07$), and frustrated ($t(28) = 1.85$; $\rho = 0.08$).

b. Negative mood responses to physical challenge

As was observed at baseline, differences existed between the depressed and control participants on anger, fatigue, depression, challenge, anxiousness, frustration, irritation, and feeling like a failure (ρ's < 0.05) prior to exercise (Table 6). No significant differences occurred between interest, in control, and vigor (ρ's > 0.05, Table 6). It is important to note that there were no significant differences within each group between baseline and pre-exercise negative moods (ρ's > 0.05) in both groups; negative mood had returned to baseline levels prior to exercise.

In response to 30 minutes after the physical challenge, the depressed participants showed increased reactivity on the following negative mood measures: fatigue ($t(27) = 2.86$; $\rho = 0.01$), decreased vigor ($t(27) = -2.08$; $\rho =$

0.05), and depressed (t(27) = 2.39, ρ = 0.03). No significant differences for reactivity were found on the following negative mood measures: anger (t(27) = 1.26; ρ = 0.23), interested (t(27) = -1.67; ρ =0.11), irritated (t(27) = 0.01; ρ = 0.99), anxious (t(27) = -0.87; ρ =0.40), challenged (t(27) = -1.27; ρ =0.22), control (t(27) = -1.18; ρ = 0.25), feel like a failure (t(27) = 0.59; ρ = 0.56), and frustrated (t(27) = -0.34; ρ = 0.74) (Table 6). These were mood assessments taken 30 minutes post-exercise, the standard time point for these types of assessments (Yeung, 1996). Immediate post-exercise negative mood was also investigated, and the depressed group showed a smaller increase in feelings of being challenged (t(27) = 3.05; ρ = 0.01), and a greater decrease in feeling like a failure (t(27) = 2.51; ρ = 0.02) than the control group (Table 6). Since the intensity level of exercise may have been different for the two groups (see description below), lactate levels were correlated with mood measures (as lactate is a measure of intensity of exercise). However, no statistically significant correlations were found (r's < 0.40; ρ's > 0.10) between lactate at peak exercise and subsequent mood measures. In addition, to control for exercise intensity, lactate was added as a covariate for the analyses presented above. None of the results were altered by the incorporation of lactate as a covariate.

V. Hypothesis 1C: Relationship between neurohormonal responses and negative mood

a. Relationship between neurohormonal responses and negative mood responses during mental challenge

The relationship between neurohormonal responses and negative mood to mental challenge was examined utilizing Pearson Correlations. Based on the typical response of neurohormones to mental challenge, correlations involving the neurohormones, ACTH, NE, and Epi were sampled immediately post-mental challenge and cortisol was sampled 30-minutes post-mental challenge. Relationships among the mood parameters are shown in Table 7. A statistically significant relationship was observed between ACTH changes and feelings of anger ($r = 0.36$, $\rho = 0.04$) and cortisol changes and feelings of depression ($r = 0.41$, $\rho = 0.03$). As the ACTH and cortisol reactivity increased, the feelings of anger and depression also increased (Table 8). A statistically significant relationship was observed between Epi changes and feelings of failure ($r = 0.61$, $\rho = 0.001$). As the Epi reactivity increased, the feelings of failure also increased (Table 8).

To examine the potential effects of group status, regressions were performed to determine if group status or neurohormonal responsiveness predicted emotional reactivity. For the relationship between ACTH and anger, neither ACTH nor group status was significantly predictive of feeling angry ($\beta_{ACTH} = 0.38$, $\rho = 0.11$; $\beta_{group} = 0.04$, $\rho = 0.88$). For the relationship between cortisol

and feeling depressed, group status was predictive of feeling depressed with cortisol changes no longer statistically significant ($\beta_{cortisol}$ = 0.10, ρ = 0.67; β_{group} = 0.48, ρ = 0.05). For the relationship between Epi and feelings of failure, neither Epi nor group status was significantly predictive of feeling failure (β_{EPI} = 0.11, ρ = 0.62; β_{group} = 0.25, ρ = 0.28). Stratified analyses for each group separately were considered, but not pursued further because only very high correlations (r = 0.70) can be detected at a power of 80% with N=14.

b. Relationship of negative mood responses to neurohormonal responses during physical challenge

For correlations involving neurohormones, ACTH, NE, and Epi were sampled at peak exercise and cortisol was sampled 30-minutes after the completion of the physical challenge. Relationships between the mood parameters are shown in Table 9. No statistically significant relationships existed between the neurohormonal responses and negative mood responses (ρ's > 0.10) (Table 10).

VI. Hypothesis 2A: Cardiovascular reactivity to challenge as related to depression

a. Cardiovascular reactivity to mental challenge

It was hypothesized that the depressed group would show increased hemodynamic reactivity. As discussed in the methods section, reactivity was evaluated as arithmetic change scores from baseline to peak level during the

mental challenge tasks (anger recall and math task). Specifically, task reactivity was defined as the peak hemodynamic response during the tasks subtracted from an average of three measurements of hemodynamic response during the rest period. Overall average, task peak, and task average responses were also examined and did not differ significantly from the peak findings presented below (Table 11). The groups did not differ at baseline on SBP, DBP, or HR (Figures 4-6).

In response to the mental challenge, the depressed participants showed increased reactivity on the following hemodynamic measures: SBP, $t(28) = 2.07$, $\rho = 0.05$ (depressed: $M = 28.8$ mmHg, $SD = 10.3$; control: $M = 20.1$ mmHg, $SD = 12.3$) (Figure 4), DBP, $t(28) = 2.03$, $\rho = 0.05$ (depressed: $M = 15.8$ mmHg, $SD = 4.8$; control: $M = 12.3$ mmHg, $SD = 4.7$) (Figure 5), and HR, $t(28) = 2.18$, $\rho = 0.04$ (depressed: $M = 16.2$ beats/minute, $SD = 7.1$; control: $M = 9.6$ beats/minute, $SD = 9.2$) (Figure 6). In order to control for the possible Type 1 error encountered by analyzing these three related measures in separate analyses, a MANOVA was conducted. The same findings were replicated with a group difference observed on the reactivity to mental stress ($F(26) = 3.83$; $\rho = 0.02$).

b. Increased cardiovascular reactivity to physical challenge

Through the course of the study, it was found that blood pressure measures were unable to be reliably obtained during the physical challenge. Therefore, the cardiovascular reactivity to physical challenge will focus on HR responses. HR responses to the physical challenge (both warm-up and peak) were subtracted from baseline HR for the change scores. Baseline measures

were used because pre-exercise HR has anticipatory increases along with the HR increases associated with posture change.

All of the participants performed the same exercise warm-up period and the depressed group showed an increased HR to this stage of the challenge, $t(27) = -2.14$, $\rho = 0.04$ (depressed: $M = 38.1$ beats/minute, $SD = 11.9$; control: $M = 29.8$ beats/minute, $SD = 8.8$) (Figure 7). The peak HR during exercise differed by groups, with the control group showing a higher peak than the depressed group, $t(27) = 2.62$, $\rho = 0.01$ (depressed: $M = 89.6$ beats/minute, $SD = 23.5$; control: $M = 108.6$ beats/minute, $SD = 14.9$). The peak heart rate during the physical challenge was changed to a percentage of age-predicted maximum heart rate (220 - age). When analyzed in this manner, there was a trend in the data suggesting the control group reached a higher percentage of maximum heart rate than the depressed group, $t(27) = 1.88$, $\rho = 0.07$ (depressed: $M = 88.4\%$, $SD = 12.4$; control: $M = 96.3\%$, $SD = 10.3$).

It is important to note that the control group had a higher percentage of individuals that completed the protocol than the depressed group, although this difference was not statistically significant ($\rho = 0.30$). The same analyses were performed on the sample of individuals that completed the exercise protocol. In this sub-group (n=13; 81% of depressed group and n=9; 64% of control group), there were no significant differences for warm-up HR, peak HR, or percentage of age-predicted maximum heart rate reached. The differences in the previous analyses therefore likely reflect consequences of the depressed group exhibiting lower exercise test performance (discontinuation of the protocol before it was

completed) than the control group (duration of exercise for control group = 19.0 ± 2.8 minutes; depressed group = 16.8 ± 4.6 minutes; ρ = 0.14).

To investigate the intensity level reached by the groups, lactate samples were analyzed. The depressed group had a lower change in lactate from pre-exercise to peak exercise than the control group, t(14) = 3.72, ρ = 0.002 (depressed: *M* = 2.2 mmol/L, *SD* = 1.4; control *M* = 5.9 mmol/L, *SD* = 2.8). In the sub-group of participants that completed the exercise protocol, the direction of the finding remained the same, but it was no longer statistically significant, t(12) = 1.90, ρ = 0.08 (depressed: *M* = 2.5 mmol/L, *SD* = 1.8; control *M* = 5.6 mmol/L, *SD* = 2.7) (se Figure 8).

VII. Hypothesis 2B: Relationship between neurohormonal and negative mood responses and cardiovascular reactivity

a. Relationship between neurohormonal responses and cardiovascular reactivity to mental challenge

A statistically significant relationship was observed between cortisol changes and HR response (r = 0.42, ρ = 0.02) (Figure 9). As the cortisol reactivity increased, HR reactivity also increased (Table 12). A statistically significant positive relationship was also observed between Epi changes and HR response (r = 0.39, ρ = 0.05) (Table 12). A lack of statistical significance existed between catecholamines (NE and Epi) and peak blood pressure measures, which is unusual. Therefore, the last blood measurement during mental

challenge was correlated with NE and Epi. This measurement was chosen because it was the closest measurement to the time of the blood draw. Again, the correlations were not statistically significant (NE-SBP r = 0.02, ρ = 0.93; NE-DBP r = 0.27, ρ = 0.43; Epi-SBP r = 0.14, ρ = 0.52; Epi-DBP r = 0.12, ρ = 0.57).

To examine the potential effects of group status, regression analyses were conducted controlling for group status. For the association between cortisol reactivity and HR reactivity, neither cortisol nor group status were statistically significant (β_{CORT} = 0.32, ρ = 0.23; β_{group} = 0.15, ρ = 0.56). For the relationship between Epi and HR reactivity, Epi and group status were no longer statistically significant (β_{EPI} = 0.29, ρ = 0.18; β_{group} = 0.22, ρ = 0.31).

b. Relationship between cardiovascular and neurohormonal responses to physical challenge

A statistically significant relationship was observed between ACTH changes and peak HR response (r = 0.59, ρ = 0.04) (Figure 10). As the ACTH reactivity increased, peak HR reactivity also increased (Table 13).

A regression was calculated to adjust for group status. For the association between ACTH reactivity and peak HR reactivity, ACTH and group status were not statistically significant (β_{ACTH} = 0.33, ρ = 0.25; β_{group} = 0.49, ρ = 0.11).

c. Relationship between negative mood and cardiovascular responses to mental challenge

The relationship between cardiovascular and negative mood responses to mental challenge was examined using Pearson correlations. A statistically

significant relationship was observed between SBP changes and feelings of challenge ($r = 0.51$, $\rho < 0.01$), depression ($r = 0.40$, $\rho = 0.03$) (Figure 11), and frustration ($r = 0.46$, $\rho = 0.01$). As feelings of challenge, depression, and frustration increased, the SBP also increased (Table 14).

To examine the potential effects of group status, regressions were calculated adjusting for group status. Between SBP changes and feelings of challenge, feelings of challenge was significantly predictive of SBP changes, while group status was not ($\beta_{challenge} = 0.48$, $\rho = 0.03$; $\beta_{group} = 0.14$, $\rho = 0.48$). For the relationship between SBP and frustration, frustration was predictive of SBP changes while group status was not ($\beta_{frustration} = 0.38$, $\rho = 0.04$; $\beta_{group} = 0.24$, $\rho = 0.19$). However, for the association between SBP reactivity and depressed mood, neither depressed mood nor group status were statistically significant ($\beta_{depressed} = 0.29$, $\rho = 0.16$; $\beta_{group} = 0.22$, $\rho = 0.28$).

d. Relationship between negative mood and cardiovascular responses to physical challenge

No statistically significant relationships were found between negative mood and cardiovascular responses to physical challenge (ρ's > 0.15) (Table 15).

VIII. Hypothesis 3A: Inflammation in response to challenge as related to depression

a. Inflammatory responses to mental challenge

It was hypothesized that the depressed group would show increased inflammatory reactivity. As discussed in the methods section, reactivity was evaluated as arithmetic change scores from baseline to peak level during the challenge tasks. Since the inflammatory factors (IL-6, TNF-α, and CRP) were not normally distributed, all analyses are performed on the natural logarithm of the factors. Geometric means and confidence intervals are presented (Table 16). The groups did not differ at baseline on IL-6 ($t(28) = 1.65$, $\rho = 0.11$), TNF-α ($t(23) = 0.38$, $\rho = 0.71$), and CRP ($t(28) = 1.05$, $\rho = 0.30$).

In response to the mental challenge, the depressed participants showed an immediate increased reactivity on the following inflammatory measures: IL-6, $t(16) = 2.42$, $\rho = 0.03$ and TNF-α, $t(20) = 2.09$, $\rho = 0.05$. As discussed in the Background section, the inflammatory response to stress can be delayed. Therefore, the inflammatory responses were also analyzed at 30 minutes after the completion of the mental challenge. The depressed participants showed an increased reactivity at 30 minutes post mental challenge on CRP, $t(25)=-2.18$, $\rho = 0.04$.

Change in plasma volume was evaluated because it can have an impact on inflammatory responses. Plasma volume changes were calculated using a method described previously (Dill & Costill, 1974). Plasma volume decreased

2.8% (SD: 20.3) in response to the mental challenge and decreased 11.6% (SD: 35.1) in response to the physical challenge. Since the depressed and control groups displayed the same amount of plasma decrease throughout the protocol (mental challenge: $t(26) = 0.39$, $\rho = 0.70$; physical challenge: $t(15) = 1.63$, $\rho = 0.12$), plasma volume will not be controlled for in subsequent analyses.

b. Inflammatory responses to physical challenge

All of the participants performed the same warm-up period and the depressed and control groups showed the same reaction to the physical challenge on the inflammatory factors: IL-6 ($t(14) = -0.54$, $\rho = 0.60$), TNF-α ($t(3) = 1.77$; $\rho = 0.18$), and CRP ($t(14) = -0.17$, $\rho = 0.86$) (geometric means and 95% confidence intervals (CI) presented in Table 17). These results were consistent for peak exercise and 30 minutes post-exercise, demonstrating that the depressed and control groups had the same pattern of inflammatory responses to the exercise challenge.

IX. Hypothesis 3B: Association of neurohormonal and negative mood responses to inflammatory reactivity

a. Relationship between neurohormonal responses and inflammation to mental challenge

For inflammatory analyses involving correlations, IL-6 and TNF-α were sampled at immediately post-mental challenge, whereas CRP was sampled 30-min after the mental challenge. Statistically significant relationships were

observed between cortisol changes and changes in TNF-α ($r = 0.56$, $\rho = 0.01$) (Figure 12) and CRP ($r = 0.44$, $\rho = 0.03$). As the cortisol reactivity increased, TNF- α and CRP also increased (Table 18).

To examine the potential effects of group status, regression analyses were calculated controlling for group status. A statistically significant relationship was observed between group status and changes in TNF- α, but not for cortisol changes ($\beta_{CORT} = 0.10$, $\rho = 0.60$; $\beta_{group} = 0.62$, $\rho = 0.01$). The same pattern was constant for the association between cortisol changes and changes in CRP, group status was statistically significant, while cortisol was not ($\beta_{CORT} = 0.01$, $\rho = 0.97$; $\beta_{group} = 0.66$, $\rho = 0.01$).

b. Relationship between neurohormonal responses and inflammation to physical challenge

For inflammatory analyses involving correlations, IL-6 and TNF-α were sampled at peak exercise, whereas CRP was sampled 30-min after the physical challenge. A statistically significant relationship was observed between ACTH changes and changes in TNF-α ($r = 0.67$, $\rho = 0.03$) (Figure 13). As the ACTH reactivity increased, TNF-α also increased (Table 19). The positive relationship between Epi reactivity and changes in TNF-α was also statistically significant ($r = 0.89$, $\rho = 0.05$) (Table 19).

Since these correlations involved less than 10 participants (8 participants data were present), the above correlations are Spearman's rho. No further analyses were performed on this type of correlation (i.e., controlling for group status).

c. Relationship between negative mood and inflammation responses to mental challenge

IL-6 increases were significantly correlated with changes in feelings of depression ($r = 0.38$, $\rho = 0.02$) and feeling like a failure ($r = 0.39$, $\rho = 0.01$) (Figure 14). As the IL-6 reactivity increased, feelings of depression and failure also increased. CRP was statistically significantly related to feeling like a failure ($r = 0.37$; $\rho = 0.03$), whereas TNF-α was related to feelings of depression ($r = 0.39$; $\rho = 0.01$) and failure ($r = 0.36$, $\rho = 0.04$) (Table 20).

To examine the potential effects of group status, regressions were calculated adjusting for group status. For the relationship between depression and IL-6, neither depression nor group status was predictive of IL-6 changes ($\beta_{depression} = 0.02$, $\rho = 0.92$; $\beta_{group} = 0.42$, $\rho = 0.06$). The same pattern held between feelings of failure and IL-6, neither feelings of failure nor group status were statistically significant ($\beta_{failure} = 0.15$, $\rho = 0.46$; $\beta_{group} = 0.37$, $\rho = 0.07$). However, for the association between feelings of failure and CRP, group status was a statistically significant predictor of CRP, whereas feeling like a failure was not ($\beta_{failure} = 0.19$, $\rho = 0.35$; $\beta_{group} = 0.48$, $\rho = 0.03$). For the relationships between feeling like a failure and TNF-α and depression and TNF- α, no statistically significant relationships existed ($\beta_{depressed} = 0.03$, $\rho = 0.93$; $\beta_{group} = 0.44$, $\rho = 0.14$ and $\beta_{failure} = 0.12$, $\rho = 0.59$; $\beta_{group} = 0.38$, $\rho = 0.01$, respectively).

d. Relationship between negative mood responses and inflammation to physical challenge

A statistically significant relationship was found between IL-6 response and fatigue ($r = 0.50$, $\rho = 0.04$) (Figure 15). The higher the emotional response 30 minutes post physical challenge, the larger the IL-6 response at peak physical challenge, the larger the increase in the (Table 21).

When examining the potential effect of group status on the association between IL-6 changes and changes in fatigue, neither fatigue nor group status was statistically significant ($\beta_{fatigue} = 0.52$, $\rho = 0.07$; $\beta_{group} = 0.04$, $\rho = 0.87$).

Discussion

I. Review of Results

This study explores the acute effects of mental and physical challenges among depressed individuals compared to non-depressed control participants in terms of mood, cardiovascular, and neuroimmunological reactivity. In particular, this study examined whether the depressed participants evidenced increased reactivity compared to non-depressed controls. In addition, the associations of negative mood and neurohormonal responses with cardiovascular and inflammatory responsiveness to the mental and physical challenges were evaluated.

In general, reactivity was higher in depressed participants as compared to non-depressed controls. The heightened reactivity was evidenced in negative

mood, neurohormonal, cardiovascular, and inflammatory parameters. More consistent results were observed in response to the mental challenge than the physical challenge. Specifically, the depressed participants showed increased responsiveness in ACTH (immediate), cortisol (thirty minutes post challenge), NE (immediate), fatigue, depressed mood, anxiousness, experiencing challenge, SBP, DBP, HR, IL-6 (immediate), TNF-α (immediate), and CRP (thirty minutes post challenge) in response to the mental challenge. In response to the physical challenge, the depressed participants showed increased responsiveness in fatigue, decreased vigor, depressed mood, and HR (warm-up period). The control group demonstrated increased reactivity to the physical challenge on ACTH (peak) and HR (peak), which may partially reflect higher workloads among controls (see below).

These results are supportive of Hypotheses 1A, 1B, 2A, and 3A. Consistent with previous research, depressed participants displayed hyper-reactivity on neurohormones, negative mood, cardiovascular, and inflammatory responses to mental and physical challenge (Deuster et al., 2000; Feldman et al., 1999; Hoaglin et al., 1983; Kibler & Ma, 2004; Lovallo et al., 1990; Stone et al., 1993; Takaki et al., 1994). However, the present investigation is the first to examine all of these variables simultaneously in the same sample of participants.

Consistently, previous research has demonstrated that higher cortisol levels in depressed individuals compared to their non-depressed counterparts (Plotsky et al., 1998; von et al., 1987). At baseline in the present investigation, no statistically significant differences occurred between depressed and non-

depressed participants. The diurnal pattern of cortisol peaks in the morning, declines throughout the day and peaks again close to midnight. Depressed individuals usually show a larger peak in the morning and at midnight (Plotsky et al., 1998). Our participants were tested between 12 pm and 4 pm, during a time period of low levels of cortisol. Therefore, differences between control and depressed participants would not be expected. In addition, atypical depression is usually associated with lower then normal levels of cortisol, but in the current investigation only one of the participants exhibited atypical depression, therefore the low levels of cortisol associated with atypical depression could not be examined. Although no baseline differences were present, the depressed participants demonstrated hyper-reactivity of cortisol in response to the mental challenge. If more atypically depressed individuals were in the investigation, the results may have been different (i.e., lower reactivity of cortisol in the depressed individuals).

The relationship between these outcome measures is a complicated issue. In response to the mental challenge, relationships existed between mood and neurohormonal responses (ACTH-anger, cortisol-depressed mood, and epinephrine (Epi)-feelings of failure), neurohormonal and cardiovascular reactivity (cortsiol-HR and Epi-HR), mood and cardiovascular responses (challenge-SBP, depression-SBP, and frustration-SBP), neurohormonal and inflammatory reactivity (cortisol-TNF-α and cortisol-CRP), and mood and inflammatory responses (depressed mood-IL-6, like a failure-IL-6, like a failure-CRP, depressed mood- TNF-α, and like a failure- TNF-α). Surprisingly, small

correlations existed between catecholamines and blood pressure (Table 12). Since catecholamines are a major determinant of the acute stress response a high correlation was expected. A previous research study examined the inter-correlation between catecholamines and blood pressure in response to mental stress (Eisenhofer et al., 1985). Catecholamine and blood pressure responses were not correlated. In addition to mental stress, Eisenhofer et al. (1985) also examined cardiovascular responsiveness to beta-adrenoceptor stimulation. Considerably improved relationships were found when cardiovascular responses were correlated with a single variable generated from the product of the adrenaline response and the inverse of the dose of isoprenaline required to raise heart rate by 25 beats/min. It was concluded that beta-adrenoceptor stimulation is an important factor in the variability between catecholamine and blood pressures responses to mental stress. Both catecholamines and adrenoceptor-mediated responses to catecholamines should be examined when determining the physiological basis for differences in cardiovascular reactivity to mental stress between individuals or groups (Eisenhofer et al., 1985). Therefore, the lack of strong relationships found in the present investigation between catecholamines and blood pressure may be explained by different sensitivities to adrenergic sensitivity. Future research should examine adrenaline responsiveness along with absolute catecholamine and cardiovascular reactivity.

In response to the physical challenge, missing data posed an unforeseen problem. Therefore, any correlations that involved less than 10 participants were conducted using the non-parametric Spearman's rho test, which is typically

associated with reduced power. In response to the physical challenge, no statistically significant relationships were found between mood and neurohormonal responses and between cardiovascular and mood responses. Statistically significant relationships were observed between neurohormonal and cardiovascular reactivity (ACTH-HR peak), neurohormonal and inflammatory reactivity (ACTH-TNF-α and Epi- TNF-α), and mood and inflammatory responses (fatigue-IL-6).

Therefore, support exists (at least in part) for Hypotheses 1C, 2B, and 3B. However, the correlations between variables do not indicate the directionality of the relationships. In Figure 1, the hypothesized direction of relationship postulates that neurohoromones and negative mood cause the cardiovascular and inflammatory reactivity. The present data neither support nor refute the directionality of these relationships. Instead, the present investigation offers evidence that relationships exist between the different systems reacting to the mental and physical challenge tasks. The correlations were not always consistent and are considered to be of moderate effect size (Cohen, 1988). Although, evidence from this investigation supports the relationship among the variables, other possibilities may exist to explain the reactivity of neurohormones, negative mood, cardiovascular, and inflammatory measures.

II. Predictiveness of Neurohormones and Negative Mood to Reactivity

A preliminary analysis was conducted to examine the predictive ability of negative mood on cardiovascular reactivity (specifically SBP) to mental challenge. The relationship between negative mood and SBP was chosen as these data are the most complete and allow the greatest power in regression analyses. The change in SBP was predicted from group status in the first step of the regression ($R^2 = 0.13$; $\beta=0.36$; $\rho=0.05$). Then the change in feelings of challenge from rest to mental challenge was entered into the regression (R^2 change = 0.14; $\beta=0.44$; $\rho=0.03$). With the addition of feelings of challenge in the regression model, group status was no longer a statistically significant predictor of change in SBP ($\beta=0.14$; $\rho=0.48$). Although this is a preliminary analysis, these findings suggest that there may be an interplay between depression status and acute task response in determining cardiovascular reactivity. However, the regression is a post-hoc analysis and should be interpreted with caution.

Adjusting for group status may not be the best solution for examining these results. It is hypothesized that depressed individuals have higher neurohormonal and negative mood in response to mental and physical challenge. Then, the neurohormonal and negative mood responsiveness causes the hyper-reactivity of cardiovascular and inflammatory measures. If group status causes the neurohormonal and negative mood responses, then controlling for group status may adversely affect the statistical analyses. The analyses basically control for the cause of the variation. However, an examination of

interaction between group by neurohormonal/emotional responses may give a more detailed examination of the results, as it would allow for determination of high versus low responders in both the control and depressed groups. Therefore, the altered reactivity shown by depressed participants may be by a sub-group of high-responders, rather than depressed individuals in general. Risk stratification can be improved by an understanding of altered reactivity shown by depressed individuals.

III. Applicability to Cardiovascular Diseased Patients

The main message supported by the current study is that depressed individuals exhibit hyper-reactivity to mental and physical challenges on neurohormones, negative mood, cardiovascular, and inflammatory measures. The results indicate that the poor health prognosis exhibited by depressed individuals may in part be related to hyper-reactivity to stressors, both mental and physical. Stressors are encountered on a daily basis and the hyper-reactivity to these day-to-day stressors by neurohormonal, emotional, cardiovascular, and inflammatory systems may partly explain the development of chronic illnesses and increased mortality observed in depression.

Depressed individuals may have hyper-reactivity to challenges for a variety of reasons. These reasons for hyper-reactivity are applicable to both cardiovascularally diseased patients and those with out cardiovascular disease. One theory on the development of depression posits that depressive symptoms

occur when cumulative stressors exceed the distress-related vulnerability threshold of the individual (Kovacs & Beck, 1978). Therefore, according to this theory, the depressed individuals in this study have already exceeded their vulnerability threshold to stressors, as symptoms are exhibited. Since the threshold has already been reached, these participants may be more reactive to any additional stressors that are encountered. In addition, depressed individuals usually have more negative expectations regarding their ability to perform tasks. These negative expectations may add additional stress to the mental and physical challenge tasks that the control participants did not experience (as the control participants' negative expectations would have been lower). Lastly, depressed individuals may have deregulated hypothalamic-pituitary-adrenal and sympathoadrenal systems caused by a lack of negative feedback regulation (Strohle & Holsboer, 2003). If the negative feedback system is not working properly, then exposure to a stressor would cause an excessive response because the response would not be dampened by negative feedback loops. Although this particular investigation did not assess vulnerability thresholds, negative expectations or the negative feedback system, previous research has demonstrated that these processes are active in individuals with depression.

Cardiovascularally diseased (CVD) patients with depression will also have the same negative expectations and deregulated stress systems as the healthy depressed participants. However, the CVD patients have concerns that may affect health outcomes in addition to hyper-reactivity. For example, medication adherence is quite low among depressed individuals with CVD (Rieckmann et al.,

2006b; Rieckmann et al., 2006a; Gehi et al., 2005). Obviously, poor medication adherence can hurt prognosis. In addition, the cardiovascular system is already compromised in CVD, which means that the lack of physical activity (a common correlate of depression) is a more important factor than in non-CVD depressed individuals. Therefore, the hyper-reactivity found in non-CVD depressed participants may be overshadowed by other factors in CVD depressed participants. A longitudinal study would help to determine the relative contributions of hyper-reactivity and other possible factors (medication adherence, physical activity, etc.) that may affect mortality rates. Hyper-reactivity is an incident reaction, whereas medication adherence and physical activity are recurrent events. The longitudinal examination would also allow for investigation of the rate of each of these types of occurrences. Interventions could then be created to reduce the factors that occur most frequently and cause the poorest outcomes.

IV. Study Limitations

a. Design

In order to document biological and psychological changes over time, a within-subjects design was used in the present investigation. One possible concern with a within-subject design involves biases in the results as a consequence of carry-over and task order effects. All of the participants

experienced a mental challenge prior to the physical challenge. Therefore, the effects found during the physical challenge may not have been independent from the delayed effects of the mental challenge. Three possible solutions for this limitation were considered. First, the mental challenge and physical challenge task could have been counterbalanced. However, this solution was not feasible when considering participant burden. If the physical challenge was to be performed before the mental challenge the recovery period between the two tasks would have been too long (approximately 2 hours) for the physiological measures to return to the baseline level. Therefore, this would have increased the laboratory time for the participants. Recovery from mental challenge usually occurs within 30 minutes. In order to be sure that mood and neurohormonal measuress had returned to baseline, the recovery period post-mental challenge was set at 60 minutes. All variables (neurohormones, negative mood, cardiovascular, and inflammatory measures) had returned to at least baseline levels prior to the physical challenge. However, the mental challenge could have affected the physical challenge.

A second way to try to modify the design would have been to perform a between-subjects study, using a 2x2 between-subjects design with participants' depression status (depressed vs. non-depressed) and type of challenge task (mental vs. physical) as the independent variables. This between-subjects design would have focused on the differences in reactivity to mental vs. physical challenges, which was not a part of the primary hypotheses. To perform the experiment in this manner would have required the sample size of the

participants to be a least double from the present project. In addition, a between-subjects design could not account for between person differences, and the within-subject design that was employed allowed each participant to serve as his/her own control.

The third solution would have been to split the one day laboratory visit into two days. Therefore, complete a mental challenge on the first day and return to complete the physical challenge on a second day (the days could be counter-balanced). The main concern with this design would be participant burden and study feasibility related to unanticipated drop out. The participants would have to have completed two full laboratory visits within a short period of time (to ensure depressive symptoms were present at both visits). Also, there would have been double the amount of participant risk and discomfort, as two indwelling catheters would have to have been inserted (one for each day). The current project enrolled depressed individuals, therefore keeping participant burden to a minimum was essential to encourage recruitment. Therefore, when considering the resources available and participant burden, the one-day within-subjects design was selected as the best method to examine the study hypotheses under these constraints.

b. Definition of Depression

Another potential limitation of the current investigation was the use of the BDI-II to define depression status. The gold standard for a clinical diagnosis of

Major Depressive Disorder (MDD) is a structured interview, such as the Structured Clinical Interview for DSM-IV (SCID) as used in this project. Two of the participants in the depressed group were not diagnosed with SCID-based MDD, which may limit the generalizablility of the study to MDD patients. When these two participants were excluded from analyses, results from the 12 participants with SCID-diagnosed MDD were essentially the same as compared with the full sample which lends validity to the use of the BDI-II as the inclusion criterion.

c. Concerns Related to Participant Enrollment

Original enrollment goals were set at a total of 50 participants, 25 depressed participants and 25 non-depressed controls. However, the recruitment of the MDD participants was difficult. Several recruitment strategies were used for the MDD participants, including: advertisements at USUHS, advertisements at the National Institutes of Health, advertisements to the general population (i.e., Washington City Paper, Washington Post Express, and Craig's List), referral from the USUHS mental health clinic, and referral from a family medicine physician. An influx of phone calls from potential participants was received using these techniques. The participants were then phone-screened for inclusion/exclusion criteria and an appointment was scheduled, if eligibility requirements were met. Approximately 25% of the phone calls received were eligible depressed participants that were scheduled for a study visit. However,

only 10% of these scheduled depressed participants arrived for their appointments. To successfully recruit 25 depressed participants at this rate, 250 participants with depression had to be scheduled, and 1,000 participants had to be phone screened. The resources needed for these recruitment demands were beyond the scope of the present study. Therefore, recruitment was stopped at 14 depressed participants, and a matching control sample was recruited. Statistical significance was found on many of the analyses performed, and therefore the smaller than anticipated sample size may not have been a major detriment to the primary aims of the study.

d. Exercise Protocol

As discussed above, the protocol was designed to take place in one laboratory visit to minimize participant burden and maximize resources available. The exercise protocol was designed to approximate 70% and 90% of VO_{2max} (see Appendix C). The participants were not pre-tested to get an absolute level of VO_{2max}, to ensure that all participants actually achieved 70% and 90% of VO_{2max} or even comparable levels of VO_{2max}. Lactate measures were obtained to provide information on intensity level achieved during the exercise protocol. As described in the Results section, the depressed and non-depressed participants had the same level of lactate during the physical challenge, indicating that the same level of intensity may have been achieved by both groups. However,

depressed participants engaged in less activity and reached lower peak heart rates during exercise.

In addition to the above issue regarding the intensity of the exercise, an unexpected issue occurred with the exercise protocol. As discussed in the Results section, a large number of depressed participants were unable to complete the entire exercise protocol. Based on the heart rate and lactate responses, the depressed participants did not appear to engage in a higher level of exertion, but they voluntarily discontinued the protocol earlier than the control participants. Because the depressed participants did not complete the same exercise protocol as non-depressed controls the results of the responses to the peak physical challenge are difficult to interpret, as the stimulus was not consistent. Therefore, a secondary analysis of participants who completed the protocol was performed. The data indicate that no statistically significant differences occurred between the two groups, but the power for these analyses was lower, since the number of participants was lower. Data also suggest that pre-screening with maximal exercise testing is generally not feasible in patients with major depression.

e. Blood Collection Procedures

The protocol for this investigation took approximately four hours of time. During the four hours, 12 blood draws were performed. Inflammatory markers were a large part of the serum analyses to be performed, therefore heparin was

not used to stop clotting that may occur with prolonged catheter insertion. Heparin is an anti-coagulant, but if injected, it could potentially bias the inflammatory serum measures. We encountered substantial difficulties with keeping the catheter active for the last hour of the protocol. Because of staffing limitations, a new catheter could not be inserted as the phlebotomist was not available after two hours into the study. Therefore, missing data on the later blood draws during exercise and post-exercise recovery were common. The missing data adversely affected the statistical power. Future studies should keep the protocol to 3 hours or under to ensure the integrity of the catheter for all blood draws.

f. Blood Pressure Assessments during Physical Challenge

The exercise protocol was originally set-up to measure heart rate and blood pressure during the physical challenge, using automated devices. We were unable to obtain adequate readings because of the movement of the participants. Efforts were made to obtain manual readings, but this was unsuccessful because of the noise of the treadmill. Therefore, the only cardiovascular measurement obtained during exercise was heart rate. In response to the mental challenge, HR was the only cardiovascular factor related to neurohormonal reactivity. Therefore, the lack of blood pressures during physical challenge may not have affected the analyses involving the relationship between neuorohormonal and cardiovascular reactivity.

V. Future Directions

In future research, special attention should be paid to the design of an appropriate physical challenge that all participants will complete. With the availability of additional resources, having the mental and physical challenges on different days would allow the catheter to be placed for only two hours at a time and allow counterbalancing of the mental and physical challenges. These two changes would facilitate interpretation of the physical challenge task and also enable complete blood sample collection.

In order to redesign the exercise protocol the goal of the research should be the main determining factor. First, if the goal is the examine the physiological systems involved in the exercise challenge then a certain level of exercise intensity must be reached (approx. 85-90% of VO_{2max}) in order to ensure all systems have been recruited to engage in the exercise challenge. For this purpose, a shorter, higher intensity exercise challenge should be designed where the participants reach peak aerobic capacity in 10 minutes or less. However, if the goal of the research is to investigate the effectiveness of exercise for mood benefits to depressed individuals, a longer protocol is necessary. Most exercise interventions for depressed individuals are approximately 20-30 minutes (Dunn et al., 2001) . Therefore, the redesigned protocol should be at a lower intensity level (approx. 55-70% of VO_{2max}) for about 20-30 minutes.

The results from this study indicate that although depressed participants are encouraged to exercise, an acute bout of exercise may cause an increase in

negative mood at 30 minutes after the cessation of exercise. Future research should focus on the supposed benefit of aerobic exercise to mood improvement. Intervention studies have demonstrated that aerobic exercise is a beneficial treatment for clinical depression (Dunn et al., 2001). However, the timeline for the effectiveness remains unknown. Depressed individuals may need to be informed that the first bout of exercise is generally not mood-enhancing, but continued exercising may be beneficial as a treatment. The experience of negative mood post-exercise may serve as a deterrent for depressed individuals to engage in exercise. In operant conditioning terminology, post-exercise negative mood serves as a punishment because increased negative mood is a negative condition. Experiencing a negative condition will decrease the particular behavior that caused the negative condition (Skinner & Morse, 1958). Thus, depressed individuals may experience negative mood (a negative condition) after engaging in exercise, this will make engaging in exercise in the future less likely to occur. It is possible if the individuals with depression are made aware of the possible negative mood reaction to exercise, adherence to exercise programs by depressed individuals would increase. However, this statement needs empirical justification.

In addition, future studies can begin to examine the direction of the relationships between neurohormones, negative mood, cardiovascular, and inflammatory reactions. The present investigation used correlation and regression analysis to determine whether relationships existed, now that

relationships have been established, the next step in the research process is to determine directionality of these relationships.

VI. Implications

The findings provide important information on the role of depression status on reactivity to both mental and physical challenges. Previous work in this area has focused on one area of consequence (i.e., cardiovascular or inflammatory), which precludes examination of the relationships between these domains of analysis. This study helps to establish the inter-relation of different physiological and emotional responses to challenge. In this investigation, depressed individuals were found to have hyper-reactivity to both mental and physical challenges. However, the health consequences of hyper-reactivity to challenges have not been examined in a longitudinal study. The longitudinal format would establish whether depressed individuals with hyper-reactivity to stressors develop health problems, including cardiovascular disease, cancer, and all-cause mortality. Interventions to offset hyper-reactivity can be constructed to reduce the adverse health consequences related to hyper-reactivity in depression. Interventions may also be an essential component of the rehabilitation process of severe Major Depressive Disorder to ameliorate the impact of the daily burden of stressors, which can be mental and physical in nature.

Tables

Table 2.

Participant Demographics.

	Control (n=16)	Depressed (n=14)
Age, *M (SD)*	38.13 (6.13)	41.71 (9.59)
Gender, No. (%)		
Female	7 (50)	8 (50)
Male	7 (50)	8 (50)
BMI, *M (SD)*	25.31 (4.16)	26.04 (3.81)
Race, No. (%)		
Asian	1 (6.25)	0 (0)
African-American	6 (37.5)	7 (50.0)
Caucasian	8 (50.0)	5 (35.7)
Other	1 (6.25)	2 (14.3)
Marital Status, No. (%)		
Single	9 (56.25)	9 (64.3)
Married	6 (37.5)	3 (21.4)
Divorced	1 (6.25)	2 (14.3)
Years of education, *M (SD)*	17.6 (2.7)	16.0 (3.3)

Income, No. (%)		
< $15,000	1 (6.25)	3 (21.4)
$15,000 - 30,000	1 (6.25)	2 (14.3)
$30,000 - 70,000	7 (43.75)	5 (35.7)
> $70,000	7 (43.75)	4 (28.6)
Job Status, No. (%)		
Full-time	12 (75.0)	7 (50.0)
Part-time	1 (6.25)	3 (21.4)
Unemployed	1 (6.25)	2 (14.3)
Retired	0 (0)	1 (7.15)
Student	2 (12.5)	1 (7.15)
Smoking Status, No. (%)		
Current Smoker	2 (12.5)	4 (28.6)
Non-smoker	14 (87.5)	10 (71.4)
Living Arrangements, No. (%)		
Lives alone	1 (6.25)	2 (14.3)
Lives with at least 1 other	15 (93.75)	12 (85.7)
Physical Activity, *M (SD)* Minutes/week	272.4 (181.1)	257.1 (166.7)
Use of Anti-Depressive Medication, No. (%)	0 (0%)	2 (14%)
Involvement in Psychotherapy, No. (%)	0 (0%)	7 (50%)

Table 3.

Neurohormonal Factors Before and After Mental Stress (MS) Challenge.

	Control			Depressed		
	Pre-MS n = 15	Post-MS n = 14	30 min Post-MS n = 15	Pre-MS n = 12	Post-MS n = 12	30 min Post-MS n = 12
ACTH pg/ml	23.1 (20 - 27)	23.6 (19 - 30)	22.7 (18 - 29)	21.8 (19-25)	24.8*[t] (22-28)	22.2 (19 - 26)
Cort µg/dl	11.4 (9 - 15)	10.8 (8 -14)	9.4* (8 - 12)	10.1 (8 - 13)	10.6 (8 - 13)	12.5*[t] (10 - 15)
NE pg/ml	332.1 (286 - 386)	357.5** (303 - 422)	----------	412.3 (326 - 521)	460.2*[t] (365 - 580)	----------
Epi pg/ml	23.2 (18 - 30)	22.3 (18 - 28)	----------	25.6 (18 - 36)	32.6*[t] (23 - 47)	----------

Geometric means (95% CI)
***** ρ < 0.05 pre versus post; pre versus 30 min post
[t] ρ < 0.05 group x time interaction (pre versus post; pre versus 30 min post)
NE and Epi not measured 30-min post-MS